University of Alberta

Investigation of *Cryptosporidium parvum* and *Giardia lamblia* in Wastewater Effluent

by

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Dedicated to

My parents,

Yiquan Huang and Yunlan Ji

For their support,

and

endless love.

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List of Symbols and Abbreviations

AIDS	Acquired Immune Deficiency Syndrome
AWWA	**American Water Works Association**
BSA	Bovine Serum Albumin
°C	Degree Celsius
CsCl	Cesium Chloride
DAPI	4',6-diamidonio-2-phenylindole stain
DBPs	Disinfection Byproducts
DI	Deionized Water
DMSO	Dimethyl Sultoxide
DNA	Deoxyribonucleic Acid
EDTA	Ethylene Diamine Tetra Acetate
FA	Immunofluorescence Assay (IFA)
FACS	Fluorescent activated Cell Sorting
FC	Faecal Coliforms
FCS	Forward Scattered Light
FITC-labelled	Fluorescein Isothiocyanate-labelled
FL1	Fluorescent Light
FS	Faecal Streptococci
g	Gram
ICR	Information Collection Rule
ID_{50}	Number of Cysts Ingested Resulting in 50% of the Test Subjects Becoming Infected
IFA	Immunofluorescence Assay (FA)
IMS	Immunomagnetic Separation
L	Litre
L/d	Litres per day
log	Logarithm
M	Mole
Min	Minute
mL	Millilitre
mm	Millimetre
mmHg	Centimetre of mercury
mW-s	milli-Watt-seconds
NTU	Nephelometric Units
(oo)cysts	Both oocysts and cysts
PBS	Phosphate Buffered Saline
PBST	Phosphate Buffered Saline Solution with 0.01% Tween20
pH	Negative Logarithm of the Effective Hydrogen Ion Concentration
PI	Propidium iodide
PVC	Polyvinyl Chloride Compound
R1, R2, R3	FACS sorting gate regions
RNA	Ribonucleic Acid
rpm	Revolutions / minute

List of Symbols and Abbreviations (Cont.)

spp.	Species
SSC	Side scattered light
TC	Total Coliforms
TSS	Total Suspended Solids
USEPA	United States Environmental Protection Agency
UV	Ultraviolet Radiation
µL	Microlitre
µm	Micron
VFA	Volatile Fatty Acids
WRPs	Water Reclamation Plants
WWTPs	Wastewater Treatment Plants
× g	Gravitational Force

1 Introduction

1.1 Wastewater treatment as it relates to public health

The objective of wastewater disinfection is to eliminate, inactivate or destroy disease-causing micro-organisms. Water carries many of the organisms that produce disease. Such organisms include *Giardia* spp., *Cryptospridium* spp., *E. coli*, *Shigella* spp., *Cholera* spp. etc. It was enteropathogenic *E. coli* that contaminated a potable water source in Walkerton, Ontario and caused the outbreak of hundreds of illness and seven of deaths in 2000. Humans obtain various diseases from micro-organisms found in water (Hurst and Murphy, 1996), not only the potable water, but also in water used for recreation and washing as well.

Among the contributors of the pathogenic contaminants found in aquatic environments, domestic wastewater seems to be one of the most important (Dowd et al., 1998). Sewage has a long history connecting it to disease, sickness and death. We often think of the surface water as pure and safe and ground water as even purer and safer. However, ground water can also cause outbreaks of wastewater related disease when it has been contaminated by sewage. Old sewers and drinking water lines with cracks or leaks can be a way for polluted water to enter the drinking water system. The public health concerns have resulted in the development of methods for treating, studying and reducing pathogens in wastewater (Acher et al., 1994).

Wastewater treatment is a relatively modern practice. Wastewater is discharged from homes, and commercial establishments, by means of sanitary sewers, which carry the wastewater. Collection systems for human wastes date back to Roman times. These

wastes were not treated but were simply discharged into the local receiving body of water. Over time, increased population and increased awareness of public health problems led to the development of wastewater treatment technologies. Concern about the control of domestic wastewater began toward the middle of the 19th century, when outbreaks waterborne diseases such as cholera were becoming common.

Wastewater treatment efforts may help to reduce the incidence of problems resulting from the discharge of wastewater into environmental surface water, including bathing zones or shellfish farming areas (Hurst and Murphy, 1996; Shieh et al., 1999). Over years, legislative restrictions have been set up in many countries for wastewater discharge into bathing zones or shellfish farming areas. Wastewater treatment will also reduce aquifer contamination, which can result either indirectly following the percolation of surface applied wastewater into the subsurface or directly during subsurface injection of wastewater (Deborde et al., 1999; Yanko et al., 1999). The treatment of wastewater is also intended to reduce the contamination of crops that may occur when wastewater is eventually discharged onto land surfaces (Acher et al., 1994). In short, wastewater treatment plants are designed to remove contaminants from water for disposal elsewhere and to produce an effluent which is harmless to public health and can be discharged to a receiving water body without causing pollution.

The designs of wastewater treatment plants can vary greatly, but they typically include five components:

1. preliminary treatment for removing large debris and material,
2. primary treatment for removing suspended solids physically,

3. secondary treatment for removing dissolved oxygen demanding wastes such as organic material,
4. tertiary treatment for removing additional pollutants such as pathogenic organisms, nutrients and etc.,
5. residuals treatment and disposal for disposing all solids removed by other processes.

This thesis, is focusing on the tertiary treatment for pathogenic organisms, which may involve various disinfection technologies.

The introduction includes six sections. In Section 1.2, the protozoan background including the biological view of *G. lamblia* and *C. parvum* and their effects on public health was briefly discussed. Section 1.3 reviews literature on the concentration of *G. lamblia* and *C. parvum* in wastewater, removal of *G. lamblia* and *C. parvum* from wastewater, pathogen viability, and the disinfection technologies involved. As the most commonly used disinfection technology in Canada, UV irradiation was introduced in Section 1.3.4, including its mechanism of disinfection and its effect on *G. lamblia* and *C. parvum*. Analytical methods for environmental detection, including collection and concentration methods, separation methods and detection methods, were reviewed in Section 1.4. In Section 1.5, the problem and objectives of this study were stated. Finally, Section 1.6 is the outline of the thesis.

1.2 Protozoan Background

1.2.1 Biological view of Giardia lamblia and Cryptosporidium parvum

G. lamblia cysts can be found in the feces of infected hosts. Trophozoites live in the small intestine of the host. They average about 15 μm in length, have a pear-shaped body with a broadly rounded anterior end, two nuclei, two slender axouemes, eight flagella in four pairs, a pair of darkly staining median bodies and a large ventral sucking disc. Cysts survive in the feces of an infected host and pass to the next host when it was ingested within food or water contaminated with feces. They average about 13 μm in length, are oval, and contain two nuclei.

G. lamblia cysts can survive up to 77 days in water at 8°C, 54 days at 21°C and 4 days at 37°C (Bingham, 1979). *G. lamblia* cysts are highly resistant to chlorine and other oxidants commonly used for water treatment (Jarroll et al., 1981). *Giardia* cysts can be inactivated if the dosage of chlorine is sufficiently high.

C. parvum is a protozoan parasite that was first recognized as a potential human pathogen in 1976. The disease became best known in immunosuppressant individuals exhibiting the symptoms now referred to as Acquired Immune Deficiency Syndrome, or AIDS (Meisel, 1976). The life cycle of *C. parvum* has six stages (Current, 1986). The active life stages live on the surface of the cells lining the small intestine, reproduce asexually, and are passed in the feces as infectious oocysts. The important stage in the life cycle is the round, thick-walled, environmentally stable oocysts, 4-6 μm in diameter. Transmission of the infection occurs via the oocysts. There is sometimes a visible external suture line on the oocysts surface, and the nuclei of sporozoites can be stained with fluorogenic dyes such as 4',6-diamidino-2-phenylindole (DAPI).

C. parvum oocysts can withstand a variety of environmental stresses, including freezing and exposure to seawater (Robertson et al., 1992). *C. parvum* oocysts have been shown to survive in cold waters in the laboratory at 4°C for up to 18 months (American Water Works Association, 1988). They are even more resistant than *Giardia* spp. cysts to oxidants, such as chlorine.

1.2.2 Health Effects

The hosts infected with *Giaridia* spp. suffer from acute diarrhoea, abdominal cramps, bloating and excessive flatulence. The severity of the illness can vary considerably from person to person and only about a quarter of infected people show symptoms of the illness. Malabsorption of food can lead to considerable weight loss and in children it can be a cause of failure to thrive. The incubation period ranges from 1 to 75 days, but on average was reported to be 7 to 10 days. Treatment with drugs can be effective, but if untreated the illness may persist for 3 to 4 years. The minimal infective dose can be as low as 1 to 10 cysts (Rendtorff, 1978; Nash et al., 1987).

The symptoms of crytopsporidiosis are diarrhoea, mild abdominal pain, nausea and vomiting, mild fever and fatigue. In persons with compromised immune systems, this parasite can cause a pronounced, chronic diarrhea. Although the disease is not usually fatal, some deaths have been attributed to cryptosporidiosis. The incubation period was reported to be between 4 and 28 days with an average of 7 days (Rendtorff, 1978; Nash et al., 1987). No drugs has been shown to be effective against *Cryptosporidium* infection and recovery from the illness was dependent on the body's immune system. DuPont and co-workers (1995) found that the infective dose varies between less than 30 oocysts to as many as 1 million oocysts, depending on the person and his physical condition.

1.3 Giardia and Cryptosporidium in Wastewater

Giardia spp. cysts and *Cryptosporidium* spp. oocysts are commonly found in sewage and surface waters and occasionally in drinking water. Various studies reported that the concentration of *Cryptosporidium* spp. oocyst in wastewater is 3.3 to 20,000/L, in surface waters receiving agricultural wastewater discharges is 0.006 to 2.5/L, in pristine surface water is 0.02 to 0.08/L, in drinking water is 0.006 to 4.8/L and in recreational water is 0.66 to 500/L (Smith, 1990). There are seasonal and geographic differences of *Giardia* spp. cysts concentration in raw wastewater (Sykora et al., 1991). In a cross-Canada survey, Wallis et al. (1995) found that 56.2% of 162 raw sewage samples contained *Giardia* spp. cysts, ranging in concentration from 1 to 88,000 cysts/L. Around 11.1% of the samples contained *Cryptosporidium* oocysts ranging in concentration from 1 to 120/L. Surveys conducted on raw and treated drinking water show that 10% of 1215 samples contained 0.001 to 2 cysts/L and 6.4% contained 0.001 to 0.005 oocysts/L (Wallis et al., 1995). Samples from the wastewater treatment plants were also examined. The arithmetic mean concentration for *Giardia* in treated wastewater was 73 cysts/100L and that for *Cryptosporidium* was 56 oocysts/100L. A study conducted in Edmonton by Goarcher and Fok (1998) showed that, between 1990 to 1996, the annual geometric mean concentration of *Giardia* cysts in raw water at two drinking water treatment plants in Edmonton, Alberta, ranged from 8 to 193 cysts/100L, and that of *Cryptosporidium* oocysts ranged from 6 to 83 oocysts/100L.

1.3.1 Concentration of Giardia or Cryptosporidium in Sewage

The (oo)cysts[1] of any intestinal protozoan parasite present in the raw sewage influent will either partition into the solid phase (the sludge) or remain with the aqueous phase (the effluent), or will be found in both effluent and sludge, no matter what treatment regime that sewage was subjected to in a plant. It would be more satisfactory if any potential pathogens were partitioned into the sludge instead of the sewage effluent, since the sewage effluent will normally be discharged into a water body which may be used for recreation, farming, domestic purposes or even as potable water supply.

In general, a number of studies suggest that in the contributory catchments for sewage influents, *Cryptosporidium* occurs less frequently than *Giardia* (Xiao et al., 2001). However, Rose and co-workers (1986) detected large numbers of *Cryptosporidium* oocysts (over 197,000/L) which was three logs higher than the *Giardia* cysts concentration in raw sewage. Madore and co-workers (1987) also found relatively high numbers of *Cryptosporidium* oocysts in sewage when analysing samples from three different locations. Between 850 and 890 oocysts/L were detected in the influent of a domestic sewage treatment plant and 5280 oocysts/L were detected in the influent of a treatment plant serving a Native American community. The highest numbers of oocysts detected (13,700/L) were not in the sewage plant influent, but in the effluent wastes from a cattle slaughterhouse.

The prevalence of *Giardia* or *Cryptosporidium* varies widely between studies conducted by different groups. When comparing these studies, it is essential to address the techniques used for sampling the sewage and the purification and subsequent

[1] The abbreviation "(oo)cyst" refers to both *Cryptosporidium* spp. oocysts and *Giardia* spp. cysts.

detection of parasite transmission stages in sewage. Some techniques may be more efficient than others. Even if the technologies are apparently very similar, quite a few other factors can lead to differences in prevalence of *Giardia* spp. cysts or *Cryptosporidium* spp. oocysts. Such factors include the relative turbidity, pH or presence of other material in the sewage effluent that may influence the recovery efficiencies, and consequently, the concentration. The concentration of *Giardia* cysts detected in sewage influent can vary significantly depending both on the time of day of sampling and upon the time of year (Casson et al. ,1990; Gassmann and Schwartzbrod, 1991).

Some research has investigated the survival of cysts and oocysts of intestinal protozoa in sewage sludge, particularly when the sludge was further treated and then utilized for economic purposes (Medhat and Stafford, 1989; Van Praagh et al., 1993; Soares et al., 1994; Whitmore and Robertson, 1995; Stadterman et al., 1995; Campbell et al., 1992). A close inter-relationship has been suggested to exist between the viability of cysts or oocysts and the type of anaerobic digester and the temperature.

Treatments of sewage sludge that are suggested be effective include mixing with animal waste and treating the combined slurry as animal waste (Yi Deng and Cliver, 1992) and freeze-thawing (Sanin et al., 1994).

1.3.2 Removal of Giardia Cysts and Cyptosporidium Oocysts in Wastewater Treatment

Removal efficiency for *Giardia* cysts has been found to vary considerably between plants and treatments utilized (Casson et al., 1990). Wastewater treatment plants that incorporate secondary treatment step often have significantly higher removal efficiencies than those with only primary settling (Robertson et al., 1995; Casson et al.,

1990). The removal efficiency for a plant that utilizes secondary treatment was reported to be as high as 99%. Removal of *Cryptosporidium* oocysts was considered to be of relatively low efficiency. However, a treatment plant with higher removal efficiency of *Giardia* cysts also has a higher removal efficiency of *Cryptosporidium* oocysts (Robertson et al., 1995).

Caution should be taken when comparing estimated removal efficiencies of treatments between studies, since the factors that influence removal efficiency can vary considerably between studies. Such factors include sampling regime used in the study, sampling approach used for sewage influent and effluent within the same study, purification technologies, and turbidities of samples, etc.

1.3.3 Pathogen Viability

It is commonly assumed that any (oo)cysts found in water are potentially infectious. However, cysts have been detected in potable water supplies in the absence of identifiable outbreaks (Lechevallier et al., 1991a; Rose et al., 1991), because it is possible that some cysts are not viable and do not pose a threat to human health. In addition, some species of *Giardia* and *Cryptosporidium* are not infective to humans. It is important that detection methods are developed that consider cysts viability and infectivity.

Cysts viability may be assessed using differential interference contrast microscopy and vital dye assays. It has been shown that uptake of the vital dye propidium iodide by cysts correlates with the inability of the parasites to excyst and infect animals (Schupp and Erlandsen, 1987). *Giardia* cysts can be excysted *in vitro* using enzymes such as trypsin at low pH and the trophozoites can be grown in TYI-S-33 medium (Diamond et al., 1978; Rice and Shaefer III, 1981). *C. parvum* oocysts can also be excysted and used

to infect bovine kidney cells *in vitro* (Upton et al., 1994). Both parasites can be used to infect experimental animals such as the gerbil for *G. lamblia* (Belosevic et al., 1983) or the neonatal CD-1 mouse (Finch et al., 1993) for *C. parvum.* Viability assessment based on vital dye (Campbell et al., 1992) inclusion or exclusion was considered to be only an estimate of infectivity. This assessment most likely overestimates viability because not all cysts classified as viable by the vital stain assay were shown to be infectious (Jenkins et al., 1997; Neumann et al., 2000). This hypothesis was confirmed by the animal infectivity test results in a study by Garcia and co-workers (2002). Determination of cysts concentrations without viability of infectivity assessment significantly overestimates the health risks associated with protozoan cysts in wastewater effluents. In the absence of a more reliable *in vitro* method to assess cyst infectivity, animal models are still considered the benchmark.

Viable (oo)cysts have been detected in the sewage influent and effluent after wastewater treatment (Smith et al., 1994). This indicates that the removal efficiency of (oo)cysts by the sewage treatment processes in place is not 100%. Furthermore, it also suggests that non-viable (oo)cysts may be selectively removed, possibly because they are more likely to attach to debris and thus settle out. The viability of cysts in effluent was frequently higher than that in the influent (Robertson et al., 1995). Presence of infectious *C. parvum* oocysts in 40% of final disinfected wastewater effluent samples was reported by Gennaccaro and co-workers (2003).

1.3.4 Disinfection Technologies

Overview

Pathogens are destroyed in significant numbers by physical and chemical treatment processes at wastewater treatment plants (WWTPs). Pathogens also die naturally, but such reduction should be distinguished from purposeful inactivation.

Inactivation strategies include heating, lighting, oxidizing agents, acids and alkalies, metal ions, and surface active chemicals (Fair, 1968). Not all disinfectants are used for treating wastewater in a single wastewater treatment plant due to the cost and other considerations. The most widely used inactivation technologies are chlorination/dechlorination, ozonation, and ultraviolet radiation (UV).

Ozone and chlorine dioxide are good chemical oxidants, but both are expensive and may result in the formation of unwanted by-products, such as mutagenic and carcinogenic agents. Compared to chlorine dioxide, ozone was usually shown to be a better choice (Rosen, 1976). However, ozone tends to be unreliable when turbidity is high or variable because cysts are protected in flocculated particles. Although, chlorination alone does not appear practical for the inactivation of *Cryptosporidium* (Finch et al., 1993), it is still used in the majority of wastewater inactivation applications. The shortcomings of chlorination and ozonation have prompted research as to seek alternative inactivation methods that would minimize environmental and public health impacts.

In North America, ultraviolet (UV) irradiation has become the most common alternative to chlorination for inactivating of pathogens in wastewater. UV was shown to be effective in inactivating most viruses, spores, and cysts. UV does not generate, handle, transport, or store toxic/hazardous or corrosive chemicals since it is a physical process

rather than a chemical oxidant. No known residual effect that can be harmful to humans or aquatic life results from UV treatment (Malley et al. 1995). In addition, UV treatment has a shorter contact time, approximately 20 to 30 seconds (Sobotka, 1993) with low-pressure mercury arc lamps. It is also user-friendly and requires less space for equipment than other methods previously mentioned.

However, there are also limitations of UV. Some viruses, spores, and (oo)cysts need a higher dose of UV for inactivation. Organisms can sometimes repair and reverse the destructive effects of UV through a "repair mechanism," known as photo reactivation, or in the absence of light known as "dark repair". A preventive maintenance program is necessary to control fouling of quartz lamp sleeves. Turbidity and total suspended solids (TSS) in the wastewater can influence the efficiency of UV inactivation. UV does not provide a residual to control pathogen proliferation and biofilm formation in the distribution system. When using UV for primary disinfection, some form of secondary chemical oxidant is required to maintain water quality in the distribution system.

Mechanism of Disinfection with Ultraviolet Radiation

The germicidal effect of UV irradiation have been recognized for many years. Specifically, UV inactivation transfers electromagnetic energy from a mercury arc lamp to the organism. The UV radiation that reaches the organism and penetrates its cell wall is absorbed by cellular materials, DNA and RNA in particular. Absorption of UV photons by the cell deoxyribonucleic acid (DNA) is believed to cause dimerization of adjacent pyrimidine nucleotide bases. Pyrimidine dimers are then formed and join neighbouring cytosine or thymine moieties by a cyclobutane ring (Bridges, 1976). These dimers are the

major cause of the lethal and mutagenic effects of UV radiation and result in preventing reproduction (Setlow, 1965; Witkin, 1976).

UV irradiation is a physical treatment and it does not alter water chemically. UV irradiation does not remove the inactivated microorganisms, inorganics or particles from the water.

Effect of UV Radiation on *Cryptosporidium pavum* and *Giardia lamblia*

Even though protozoa were once considered resistant to UV radiation, recent studies have shown that ultraviolet light was capable of inactivating protozoan parasites (Bukhari et al., 1999; Clancy et al., 1998; Craik et al., 2000).

Campbell and co-workers (1995) used a low-pressure ultraviolet light system with a theoretical minimum intensity of 14.58 mW/cm^2 and a contact time of 10 minutes, which caused a 2 to 3-log reduction in the viability of *C. parvum* oocysts. For human-derived *G. lamblia* cysts, Campbell and co-workers (2002) demonstrated that a UV dose of 10 mJ/cm^2 resulted in significant inactivation of the *Giardia* cysts. The infection kinetics of UV exposed cysts were directly compared to the infection kinetics of control (non UV exposed) cysts. A UV dose of approximately 10 mJ/cm^2 resulted in up to 2 log (99%) inactivation and a higher UV doses between 20 to 40 mJ/cm^2 resulted in up to 3 log (99.9%) inactivation of the cysts.

It has previously been reported that *G. lamblia* cysts are resistant to UV irradiation at the doses delivered by commercial systems (Rice and Hoff, 1981; Karanis et al., 1992; Karanis et al., 1994). However, this conclusion was based upon data generated by using a *in vitro* excystation for assessment of viability. However, Craik et al. (2000) demonstrated that 2 to 3 log inactivation of *Giardia muris* cysts could be achieved

with UV doses from 5 to 83 mJ/cm^2 using a C3H HeN mouse model and a medium pressure UV light source. Recent research demonstrated that protozoa such as *G. lamblia* and bacteria have similar, high sensitivity to UV light (Mofidi et al., 2002). In the study, the infectivity of *G. lamblia* and *G. muris* was determined by Mongolian gerbils and CD-1 mice respectively, and a >2-log_{10} (99%) inactivation was observed at a UV dose of $3mJ/cm^2$.

In vitro excystation is not a reliable indicator of the viability of *G. muris* cyst after UV disinfection (Neumann et al., 2000; Hayes et al., 2003). Hayes and co-workers compared *in vitro* excystation and animal infectivity in the study which assessed cysts viability after exposure to low-pressure UV irradiation. A reduction range from 0.3 log_{10} to 4.4 log_{10} with UV dose at 1.4, 1.9, 2.3 mJ/cm^2 respectively was observed in this study. Ironically, a UV dose as high as 200 mJ/cm^2 did not prevent some cysts from excysting.

1.4 Analytical Methods for Environmental Detection

The methodology for the detection of *Cryptosporidium* oocysts and *Giardia* cysts in water is completely different from that traditionally used for quantification of fecal indicator bacteria in the water industry. The analysis of a water sample for the presence of oocysts and cysts consists of three stages: sample collection and concentration, separation of (oo)cysts from contaminating debris, and detection of (oo)cysts. The routine analysis of protozoan parasites in water samples relies upon direct microscopic detection after concentration of particulate matter by filtration or centrifugation.

A typical analysis procedure using a filter for sampling is as follows. By filtration, back flushing, rinsing, hand washing or machine processing, particulate matter is recovered and concentrated into a pellet. The background material in the pellet is then

purified by discontinuous density gradient centrifugation or by IMS (immunomagnetic separation). Consequently, denser particles are separated through the density medium and form a pellet at the bottom of the tube. *Giardia* spp. cysts and *Cryptosporidium* spp. oocysts will float on the surface of the medium and are recovered by pipette. The recovered material is then concentrated by centrifugation, and the final pellet examined microscopically.

It is difficult to compare the effectiveness of methods used in different laboratories since many factors may have significant effects on the overall efficiency of recovery, such as water quality and age of the (oo)cysts. Current method for drinking water (treated or untreated) is USEPA 1623 which has been used for wastewater as well.

1.4.1 Collection and Concentration Methods

Many methods of concentrating water samples to detect the presence of protozoan parasites have been evaluated for their efficiency and suitability. These include membrane filtration, cartridge filtration, cross-flow filtration, vortex-flow filtration, flocculation, density centrifugation, and continuous-flow centrifugation. No single method has been found to be suitable for all possible situations. The choice of method should be made with due regard to a number of factors, including the purpose of sampling, the water quality, the facilities in the laboratory that will perform the analysis, the site of sample collection, and the distance over which samples must be transported. A good method is one that can efficiently concentrate as large a sample of test sample as possible and yield a concentrate that can be examined easily. It is often recommended to take a large number of low-volume samples (e.g. 10-20 liters) to be examined.

Sample concentration can be accomplished by filtration. Several types of filtration have been developed over the years. These include the 1-μm nominal porosity wound filter (Jakubowski and Ericksen, 1978), the 2-μm absolute porosity membrane filter (Wallis and Buchanan-Mappin, 1984; Sparlding et al., 1983; Ongerth, 1989) or the 1-μm absolute porosity polysulphone filters (Fricker and Clancy, 1998). Recovery efficiencies for polysulphone filters ranging from 70 to 80% have been reported. Membrane filters offer higher recovery efficiencies, but the amount of water sample that can pass through without filter clogging is relatively small, often only 10 to 20 L.

The first reported method for detection of *Giardia* spp. and *Cryptosporidium* spp. in water used polypropylene cartridge filters, with a nominal pore size of 1 μm, through which large volumes of water (100 to 1000 litres) were passed at a flow rate of 1 to 5 litres/minute. Trapped material was then washed and the resulting washings from these cartridges required further concentration by centrifugation. The recovery of *Cryptosporidium* oocysts by this technique was originally reported to be 14 to 44% (Musial et al., 1987), although lower efficiencies (1 to 30%) have often been reported (Ongerth and Stibbs, 1987; Clancy et al. 1994; Shepherd and Wyn-Jones, 1996). Differences in the reported recovery rates may be due to non-standardized factors including water quality, laboratory efficiency, and (oo)cyst age.

Membrane filtration introduced by Ongerth and Stibbs (1987) used large diameter (142 or 293 mm), 2 μm absolute, flat membranes for the concentration of oocysts from water samples. The recovery efficiency is 9% for *Cryptosporidium* and 49% for *Giardia* (Nieminski, et al., 1995). Different membranes in terms of size and material can influence the recovery efficiency (Shepherd and Wyn-Jones, 1996).

Another established method for concentrating (oo)cysts is the calcium carbonate flocculation procedure (Vesey et al., 1993b). Recovery efficiencies using this method have been reported to be as high as 70% for both *Cryptosporidium* and *Giardia* (Vesey et al., 1993a; Campbell et al., 1994; Vesey et al., 1994; Shepherd and Wyn-Jones, 1996).

1.4.2 Separation Methods

The most widely used separation methods are density centrifugation, immunomagnetic separation (IMS), and Fluorescence-activated Cell Scanning/Sorting (FACS). All of these methods have been used to isolate parasites from wastewater samples in the research described in this thesis.

Density centrifugation

Density centrifugation is often used to separate (oo)cysts from background debris and thus reduce the amount of material to be examined. However, when sucrose flotation was used for which parasites the recovery efficiency decreased greatly (Fricker, 1995)

IMS

IMS is a more efficient method for separation of (oo)cysts from other particulates. It has been used in conjunction with the 1-µm absolute filters. After filtration, the collected material was eluted with a detergent and concentrated by centrifugation. The pellet was re-suspended in buffer and mixed with monoclonal antibodies specific to *C. parvum* or *G. lamblia* attached to magnetized particles. The (oo)cysts are then separated from the debris using a magnet.

The technique is very simple to operate and it efficiently separates (oo)cysts from other particulates, such as autofluorescing algae, that cannot be removed by density

centrifugation. On the other hand, the monoclonal antibodies used are not necessarily specific to the species of parasite (Campbell et al., 1997).

When IMS is used and beads are mixed with water concentrates, the immunoglobulin-(oo)cyst complex subjected to shear forces. The stronger the bond, the more likely the bead is to remain in contact with the (oo)cyst and thus the higher recovery efficiency. The use of antibodies specific for *Giardia* spp. cysts and *Cryptosporidium* spp. oocysts greatly enhanced the probability of finding the organisms against background material and of making a positive identification. However, if attachment of antibodies to the bead is weak, the antibody may detach and the (oo)cyst will not be recovered. The most critical factor associated with the recovery efficiency of IMS is the turbidity of the water concentrate. (Oo)cysts are recovered efficiently when they suspended in relatively clean water (Campbell and Smith, 1997, Campbell et al., 1997, Fricker and Clancy, 1998). Under the conditions of these studies, the reported recovery efficiency was greater than 90%.

A number of commercial IMS technologies have been developed, including the MPC separator series by Dynal AS and Trondheim of Norway, the MACS system by Miltenyi Biotec GmbH and Bergisch Gladbach of Germany, and the Immunicon by Huntington Valley of PA.

1.4.3 Detection Methods

Epifluorescence microscopy was used to detect *Cryptosporidium* oocysts and *Giardia* cysts by examining material deposited on multi-well slides or membrane filters. The (oo)cysts are specifically stained with monoclonal antibodies which have been conjugated to FITC. Several anti-*Cryptosporidium* and anti-*Giardia* antibodies are

commercially available, but there is no single antibody that seems preferred for all purposes. Some antibodies, including commercially available antibodies, fail to specifically identify *C. parvum* or *G. lamblia* because they apparently cross-react with other species in the genus.

Flow cytometry and FACS

The specialized equipment and technical skills involved in the detection of *Giardia* cysts and *Cryptosporidium* oocysts by immunofluorescence microscopy are quite demanding. Alternative techniques for detecting (oo)cysts following concentration and purification have been proposed. For example, flow cytometry and cell sorting can automate the recovery process but require expensive and specialized equipment (Fricker and Clancy 1998).

Flow cytometry has been attempted using environmental samples to detect *Cryptosporidium* oocysts, though it was found that instruments were not sufficiently sensitive to distinguish oocysts from background material (Vesey et al., 1991). Vesey and co-workers (1993a) claimed that the incorporation of cell-sorting enabled oocysts to be sorted efficiently from background material. This technique seems to work equally well for *Giardia* cysts (Vesey et al., 1994; Medema et al., 1998a). As a first step of this separation procedure, water concentrates are stained in suspension with fluorescein isothiocyanate-labelled (FITC-labelled) antibodies and passed through the FACS. Immunofluorescent staining is usually performed by trapping a portion of the pellet on a small membrane and rinsing antibodies through, but it can also be carried out in centrifuge tubes or on microscope slides (LeChevallier et al., 1991a; LeChevallier et al., 1991b; Sauch, 1985; Wallis, 1994). Particles with the fluorescence and light-scattering

characteristics of (oo)cysts are then counted by flow cytometry, sorted from the sample stream, and collected on a microscope slide or membrane filter. Finally, epifluorescence microscopy is used to examine the particles to confirm the presence of (oo)cysts.

Several groups from France, the Netherlands, and the United States (Danielson et al., 1995; Compagnon et al., 1997; Medema et al., 1998a) have confirmed the benefits of FACS procedure by examining the presence of (oo)cysts in water samples. It has been widely used in the United Kingdom for water analysis and is becoming more widely adopted in other parts of Europe, in Australia, and in South Africa. However, the FACS procedure is not sensitive sufficiently to give a definitive indication of the number of (oo)cysts present. It can not distinguish (oo)cysts from other organisms and particles of similar size (e.g. autofluorescent algae) that may cross-react with the monoclonal antibody and have similar fluorescence characteristics. However, the confirmation by epifluorescence microscopy can be performed much more easily and reliably than direct microscopy of non-sorted samples.

1.5 Problem statement and objectives

C. parvum and *G. lamblia* are two pathogenic protozoa that are of human health concern worldwide due to their virulence, widespread occurrence, resilience to microbial reduction methods and environmental conditions. Many studies carried out in the past have shown that the prevalence of *C. parvum* and *G. lamblia* present in wastewater effluents are often high. It is well known that *C. parvum* and *G. lamblia* are resistant to chlorination/dechlorination inactivation. As an alternative, UV irradiation has been widely used at WWTPs in Canada to inactivate *C. parvum* and *G. lamblia* in wastewater effluents. It has been reported that UV treatment was effective against *C. parvum* and *G.*

lamblia under well controlled laboratory conditions. However, little is known regarding the effectiveness of UV treatment reactors operating under field conditions for inactivation of *C. parvum* and *G. lamblia* present in wastewater effluents. The infectivity of *C. parvum* oocysts and *G. lamblia* cysts detected in UV treated wastewater effluents and their significance to public health has rarely been studied.

This project therefore had two objectives:

1. To evaluate the methods used by Michener (2002) for concentrating, enumerating and assessing the infectivity of *C. parvum* oocysts and *G. lamblia* cysts in UV treated wastewater effluents. Using the Miltenyi Biotech IMS system with flow cytometry/FACS detection, Michener (2002) successfully detected *C. parvum* oocysts and *G. lamblia* cysts in treated and untreated drinking water. The difference between drinking water and wastewater is that the concentration of suspended solids and dissolved organic content in wastewater tends to be much higher than that in drinking water, which creates additional challenges for parasites recovery and purification. On the other hand, the prevalence of parasites, and *G.lamblia* in particular, was expected to be significantly greater in wastewater than in drinking water, which increased the likelihood of recovering sufficient parasites for assessment of their infectivity using animal models.

2. To determine the infectivity of *G. lamblia* cysts in UV-treated wastewater effluents at two municipal wastewater treatment plants in Western Canada using the Mongolian gerbil model.

1.5.1 Hypotheses

G. lamblia and *C. parvum* should be inactivated by UV at wastewater treatment plants. Therefore the hypotheses of this study were:

1. No gerbil inoculated with wastewater concentrates collected from downstream of the UV reactors should score positive for infection in the infectivity analysis.

2. Some gerbils inoculated with wastewater concentrates collected from upstream of the UV reactors should score positive for infection in the infectivity analysis.

To test these hypotheses, wastewater concentrates were collected from both upstream and downstream of the UV reactors at the two wastewater treatment plants. Methods used in previous studies for drinking water in which an animal model such as Mongolian Gerbils and CD-1 mice were used to test the infectivity of *G. lamblia* and *C. parvum* were adapted and modified wherever necessary. The modified method was used to test the effectiveness of UV treatment at full-scale WWTPs for reducing the viability of *G. lamblia* and *C. parvum* in wastewater.

1.5.2 Experimental Approach

In this study, the Miltenyi Biotech IMS system and flow cytometry/FACS detection was adapted to detect *G. lamblia* and *C. parvum* in wastewater effluents from the WWTPs in City of Edmonton and City of Kelowna. The modified method consistes of three key components, filtration, followed by IMS or sucrose flotation and then FACS. In this study, a 150 L of wastewater sample at Goldbar WWTP in City of Edmonton and a 600 L of wastewater sample from City of Kelowna were collected. Samples were then eluted through the filter, and a portion of the eluates were analyzed for infectivity using the Mongolian gerbil model. The remainder of the eluates were separated by IMS, enumerated by FACS and counted by microscopy. We hoped that FACS, unlike microscopy, increased the sensitivity of (oo)cyst detection, because it enable an entire water sample concentrate to be analyzed (Hoffman et al., 1997; Belosevic et al., 2000).

1.6 Thesis Overview

In Chapter 2, the methodology used for *C. parvum* and *G. lamblia* detection and infectivity analysis was described. The Miltenyi Biotech IMS system with flow cytometry/FACS detection used by Michener (2002) was modified to suit for determining the infectivity of *C. parvum* and *G. lamblia* in treated municipal wastewater. The IMS system was replaced by the sucrose flotation purification and FACS counting was replaced by microscope counting to detect *Giardia* cysts. Reasons for these modifications are explained.

In Chapter 3, the Miltenyi Biotech IMS system with flow cytometry/FACS detection was evaluated for recovering and enumerating cysts and oocysts present in treated municipal wastewater at the two WWTPs in City of Edmonton and City of Kelowna. The recovery of prepared *C. parvum* oocysts spiked into deionized water is measured using the Miltenyi Biotech IMS system with flow cytometry/FACS detection. The recovery of prepared and immunofluorescently-labelled *G. lamblia* cysts was measured using a method developed in Chapter 2 where the Miltenyi Biotech IMS purification procedure was replaced with sucrose flotation. Recovery rates are compared with those reported in the literature. Possible explanations for low recovery rates are discussed.

In Chapter 4, the infectivity of *G. lamblia* cysts in UV-treated wastewater effluents at two municipal wastewater treatment plants, Goldbar WWTP and the WWTP in City of Kelowna was determined using the Mongolian gerbil model. The effectiveness of full-

scale UV reactors at these two study sites were measured. Findings of infections detected in wastewater samples collected from the study sites were discussed.

Finally, Chapter 5 summarizes the main contributions of this thesis to the literature and concludes. Some recommendations are made for the wastewater treatment practitioners based on the studies.

2 METHODOLOGY

2.1 EXPERIMENT DESIGN

The experimental protocols for recovery and concentration of (oo)cysts for this project was adapted from that of Michener's M. Sc. thesis (2002). The Miltenyi Biotech IMS system with flow cytometry/FACS detection used by Michener (2002) was modified for determining the infectivity of *C. parvum* and *G. lamblia* in treated municipal wastewater.

Figure 2.1 demonstrate the sequence of operations involved in this study. The treated wastewater samples were collected from WWTPs and filtered on site. After filtration, the samples were eluted at University of Alberta. Sample concentration was then done using membrane filtration. Michener (2002) successfully determined the infectivity of *C. parvum* and *G. lamblia* in untreated drinking water using the IMS separation followed by FACS enumeration. However, when the IMS system was applied on treated wastewater samples, the IMS separation column was often plugged by the particles in wastewater sample and failed to separate the *C. parvum* and *G. lamblia*. The 1M sucrose flotation purification was used instead. Because *C. parvum* oocysts clump present in wastewater, FACS counting was replaced by microscope counting to detect *Cryptosporidium* oocysts and *Giardia* cysts. There are many commercial antibodies available, but they are genus specific only (Campell et al., 1997). In this study, the immucell and anti-*Giardia* antibody (kind gift of Dr. G. Faubert, professor from Institute of Parasitology, McGill University) were used to stain *C. parvum* and *G. lamblia,*

respectively. The Mongolian gerbil model was then employed to examine the infectivity of cysts.

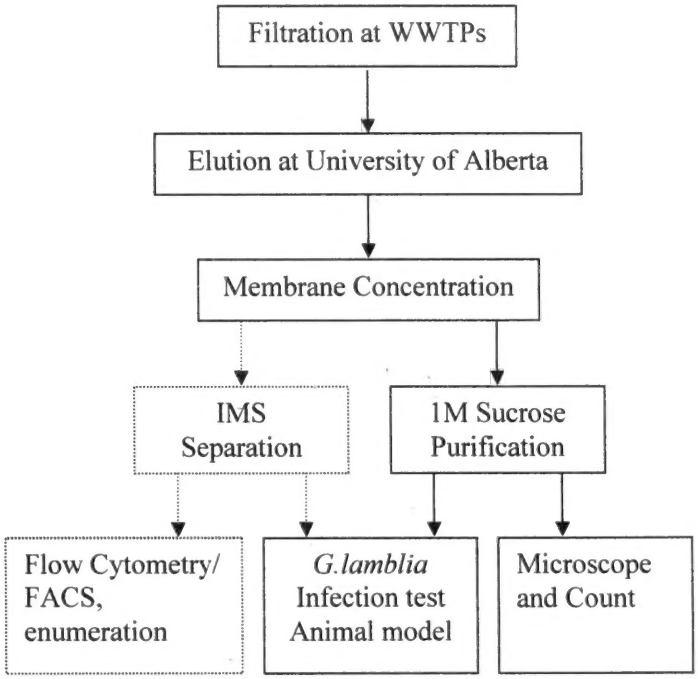


Figure 2. 1: Procedural flow diagram

In the initial stage of this project, a series experiments of purifying (oo)cysts from wastewater were carried out by using MACS system which had been used by Michener (2002) who had successfully separated (oo)cysts from untreated water samples.

2.1.1 Filtration

In this study, the Filta-Max™ system (IDEXX Laboratories, Inc.)(Protocol for sampling see Appendix 7.4) that has been specifically designed to optimize both the capture and recovery of the waterborne pathogens *Cryptosporidium* and *Giardia*, from

treated and untreated drinking water was used to concentrate these parasites from wastewater samples. The filter was made of open-cell foam discs that have been compressed to give a nominal pore size of 1μm for the capture of *Cryptosporidium* and *Giardia*. After sampling, the foam was decompressed, enabling the captured organisms to be recovered by a simple washing and concentration procedure.

About 1000 liters of treated drinking water or 50 liters of untreated water were sampled with one filter. The particulate matter in samples can be concentrated to 25 mL without the need for centrifugation. The filter operates at sampling flow rates from 1 to 4 liters per minute.

Basic steps for using Filta-Max™ filtration procedure was described in Figure 2.2.

(a). Connect the housing to the required water source for sampling

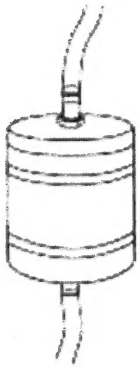
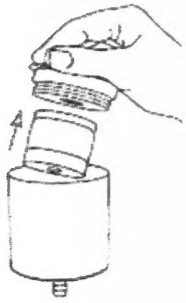
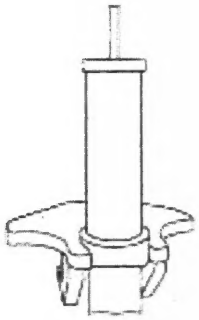


Figure 2. 2: Basic steps for using Filta-Max™ filter (written permission from IDEXX was obtained for using images in Figure 2.2)

(b). Following sampling remove the filter module from the housing



(c). Place the module in a wash station and wash with elution buffer (PBS)



(d). Concentrate the wash buffer using a 3-μm membrane

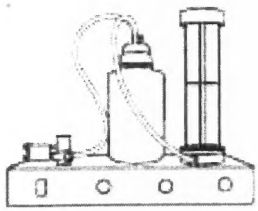


Figure 2. 3: Basic steps for using Filta-Max™ filter (written permission from IDEXX was obtained for using images in Figure 2.2)

(e). Repeat steps 3 and 4 to give a final concentrates

Figure 2. 4: Basic steps for using Filta-Max™ filter (written permission from IDEXX was obtained for using images in Figure 2.2)

Filtration at the Goldbar Wastewater Treatment Plant in City of Edmonton

Edmonton Goldbar Wastewater Treatment Plant serves over 640,000 people in Edmonton and surrounding areas. It treats approximately 100 billion litres of wastewater each year. The influent of Goldbar Wastewater Treatment Plant includes domestic sewage, groundwater infiltration flows, street runoff from some older areas of the city, and wastewater from commercial, industrial and institutional establishments.

The principle benefit of natural treatment which has been used in Goldbar Wastewater Treatment Plant is the protection of fish and other organisms such as the plants in the river. Without adding chemicals, the treatment process in Goldbar Wastewater Treatment Plant includes primary treatment, secondary treatment, and tertiary treatment.

High intensity ultraviolet light which can produce a 99.9% reduction in coliform bacteria has been used at Goldbar Wastewater Treatment Plant to inactivate the final effluent since 1997. There are four UV light channels in the Goldbar Wastewater

Treatment Plant. Each channel contains two banks of 90 high-intensity, self-cleaning UV lamps.

Equipped with variable output, high intensity lamps, and fully automated AntiClean system, the Trojan 4000 UV reactors installed in Goldbar WWTP distinguishes itself from Trojan 3000 UV reactors which has been used by City of Kelowna. More than 90% lamps could be reduced by using high intensity lamp. As a result, space requirement and installation cost will be reduced too.

About 150 L of secondary effluent wastewater both upstream and downstream of the UV reactor were filtered by IDEXX Filta-max™ filters at a flow rate between 1 and 4 L/min. The sample locations for upstream UV reactor and downstream UV reactor were located inside the UV plant building at Goldbar WWTP. The field sampling apparatus, described in Figure 2.3, consisted of a sampling tube (3 m length, 15 mm O.D. rigid PVC), a portable utility jet pump (Simer Model 2825ss, Rival Company, USA) which need to be primed before the start of filtration, a PVC ball valve, a pressure gauge (0~200psi), and a water meter (ABB, USA), and the Filta-Max filter. System components were connected with Tygon™ tubing. The end of the sampling tube was inserted into the wastewater channel. The ball valve was used to regulate the flow rate and to ensure that the pressure at the inlet of the filter did not exceed the manufacturer's recommended limit of 8 bars. About 150 L of wastewater ran through the system (minus the filter) prior to each filtration.

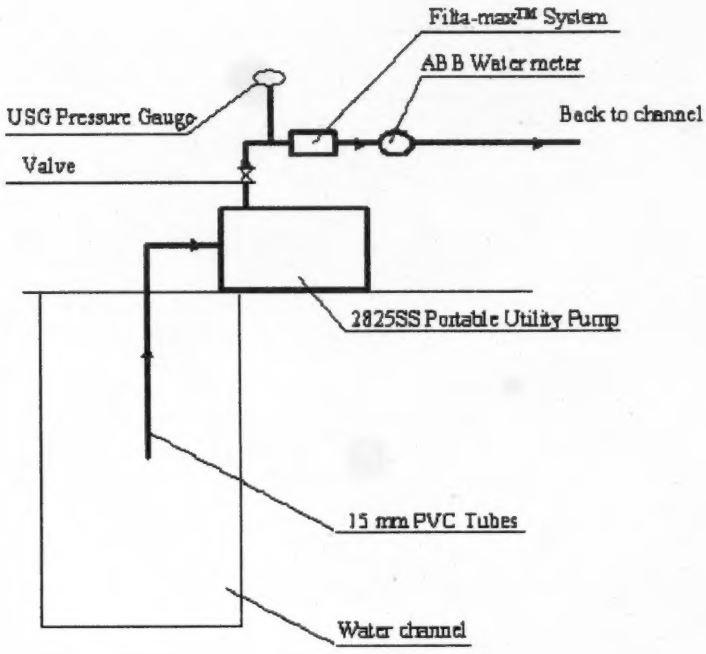


Figure 2. 5: Sampling apparatus at the Goldbar WWTP in City of Edmonton

Following filtration, the filter housings were removed from the sampling apparatus and the inlet and outlet were sealed with rubber stoppers. The filters were then sealed in plastic bags and were transported in coolers to University of Alberta laboratories. The filters were stored at 4°C before being processed. Samples were processed within 24 hours of collection to minimize the loss of parasite infectivity.

Filtration at the WWTP in City of Kelowna

The wastewater treatment process in City of Kelowna consisted of primary clarification, a biological nutrient removal activated sludge system, dual media gravity filtration and UV disinfection.

A Trojan 3000 UV reactor equipped with low pressure lamps was introduced in 1989. Because the UV lamps are installed horizontally parallel to the flow format, the Trojan 3000 UV reactor can produce a more effective dose delivery than the other low pressure UV lamp systems. Due to the use of a tertiary sand filter, the quality of the secondary effluent at the WWTP in City of Kelowna was higher than that at Goldbar WWTP in City of Edmonton (the total suspended solids at Goldbar WWTP is 5 mg/L and that at the WWTP in City of Kelowna is 0.9 mg/L). For the same reason, it was suspected that the concentration of parasites would be lower at the WWTP in City of Kelowna compared to that at Goldbar WWTP. Therefore, the sampling volumes at the WWTP in City of Kelowna were increased. About 600 L of secondary effluent wastewater samples were filtered by a Filta-Max™ filter at a flow rate between 2 and 7 L/min both upstream and downstream of the UV reactors (Trojan 3000, Trojan industries). The field sampling apparatus (Figure 2.4) consisted of the hoses (15 mm), a submersible pump (Gould, ½ horsepower), the Filta-max™ filter, a Schlumberger flowmeter, and a pressure gauge (Nuova/FIMA). Note that at the WWTP in City of Kelowna, the submerside pump was inserted directly into the channel. About 600 L of wastewater ran through the system (minus the filter) prior to each filtration.

The wastewater samples from the WWTP in City of Kelowna were collected by Marcia Browne (Technician, City of Kelowna). After filtration, filter housings were

plugged, placed in Zip Loc plastic bags and transported in coolers. They were shipped by air courier and were stored at 4°C at University of Alberta before being eluted.

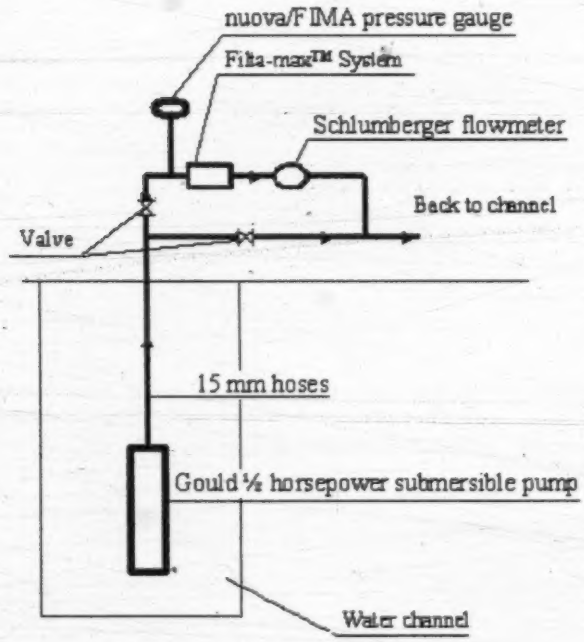


Figure 2. 6: Sampling apparatus at the WWTP in City of Kelowna

2.1.2 Elution

The filters from both Goldbar WWTP and the WWTP in City of Kelowna were eluted according to the methods outlined in Bukhari and Clancy (2000) and Filta-Max™ Protocol manual. The time period between filtration and completion of elution was longer for the WWTP in City of Kelowna due to the overnight shipping requirement. In order to gather sufficient viable (oo)cysts for the infectivity test, the whole processes of filtration and elution were completed within 72 hours.

A 3 µm cellulose nitrate membrane was placed on the concentrator column base under 600 mL PBST (phosphate buffered saline solution with 0.01% Tween® 20). The screw holding the filter's compressed foam pads was screwed onto the plunger head. The elution column was assembled and the screw was released by an Allen key, allowing the foam pads to decompress and expand. The foam pads were then gently plunged up and down in PBST 20 times. The concentrator column was removed from the plunger manifold, the plunger was pumped to empty the stainless steel tube and the concentrator column was placed on a magnetic stir plate. The elute was drained through the membrane filter with a pump and a maximum transmembrane pressure of 300 mm Hg until approximately 20 mL of the elute remained. The excess solution was poured off into a beaker and the process was repeated with 10 plunges for the second wash.

When either the membrane filter was plugged or the elution process was complete, the membrane will be washed by hand by kneading twice in a zip-loc bag with 10 mL PBST for 1 minute per washing. The sample was pipetted into the beaker that contained the excess elute. The elute was then transferred into 50 mL centrifuge tubes for further processing.

If the membrane filter was plugged and the elution process was not completed, the remaining wastewater sample was transferred to a container. The plugged membrane was replaced and concentration was continued. The elutes from each membrane were combined.

2.1.3 *Detection and Enumeration of Parasites Using IMS and Cytometry and Fluorescence-Activated Cell Sorting (FACS)*

Immunomagnetic Separation

1. *C. parvum*

The objective at the outset of this study was to use the Miltenyi Biotech MACS IMS system coupled with flow cytometry with fluorescence activated cell sorting (FACS) attempt to purify and enumerate *C. parvum* oocysts and *G. lamblia* cysts present in wastewater concentrates and in spiked controls. This approach was used successfully by Michener (2002) to recover oocysts and cysts from natural surface water and treated drinking water. Due to difficulties with this procedure encountered in the study, discussed later, only a limited number of experiments were completed with the IMS and flow cytometry. These experiments were done using either prepared *C. parvum* oocysts suspended in deionized water, or using naturally occurring *G. lamblia* cysts present in wastewater concentrates.

The principle of MACS technology is based on the use of MACS MicroBeads, MACS Columns and MACS Separators. MACS MicroBeads are superparamagnetic particles that are coupled to highly specific monoclonal antibodies. They are used to magnetically label the target cell population. *C. parvum* oocyst flow cytometry samples underwent immunomagnetic separation (IMS) using Miltenyi Biotech MiniMACS columns (Cat#130-042-201) and MicroBeads (Cat#130-048-701). Oocyst samples were centrifuged in 15 mL centrifuge tubes for 15 minutes at 1400×g. The supernatant was aspirated down to 0.5 mL and the pellet was re-suspended in 9.5 mL IMS buffer and incubated for 30 minutes at room temperature. Ten-µL anti-*Cryptosporidium parvum*

antibody (Cat# LR50, ImmuCell, 56 Evergreen Drive Portland, ME 04103) was added. The suspension was incubated for 30 minutes at room temperature then centrifuged at 1400×g for 15 minutes. The volume was reduced to 0.5 mL. Twenty µL of MicroBead solution was added and the suspension was re-suspended. The sample was incubated at 4°C for 20 minutes.

After placement on the MiniMACS magnet, the MACS Separation Column and Pre-Separation Column (Cat#130-041-407) were rinsed with 500 µL IMS buffer. The suspension was pipetted into the pre-separation column and the centrifuge tube was rinsed three times with 500 µL IMS buffer. The pre-separation column was rinsed with 500 µL IMS buffer and the MiniMACS separation column was rinsed twice with 500 µL IMS buffer. The MACS separation column was then removed from the magnet and flushed 5 times with the plunger using 500 µL IMS buffer per flush.

The resultant positive fraction was then centrifuged at 1400×g for 15 minutes, reduced to 0.5 to 1 mL, and stored at 4°C before the flow cytometry and FACS analysis.

2. *G. lamblia*

Wastewater concentrate samples were centrifuged at 388×g for 10 minutes and the volume was reduced to 0.5 mL. The cysts were re-suspended in 5 mL PBS and 5% calf serum (Atlanta Biological Cat# S11250). The suspension was incubated for 15 minutes at room temperature. One mL anti-*G. lamblia* monoclonal antibody hybridoma supernatant (kind gift of Dr. G. Faubert, professor from Institute of Parasitology, McGill University) was added and the suspension was incubated for 30 minutes at 37°C. Two-µL anti-mouse Ig polyclonal (Pharmigen, Cat#12064D) was added. The sample was then

incubated for 30 minutes at 37°C. The suspension was then centrifuged at 388×g for 10 minutes and aspirated down to 0.5 mL. Finally 20-µL of MicroBeads were added and the solution was incubated at 4°C for at least 20 minutes.

Following placement on the MiniMACS magnet, the MiniMACS Separation Column and Pre-Separation Column were rinsed with 500 µL IMS buffer. The cysts suspension was pipetted into the pre-separation column and centrifuge tube was rinsed three times with 500 µL IMS buffer. The pre-separation column was then rinsed with 500 µL IMS buffer and the MiniMACS separation column was rinsed twice with 500 µL IMS buffer. The separation column was then removed from the magnet and flushed five times with the plunger using 500 µL of IMS buffer per flush.

The resultant positive fraction was then centrifuged at 388×g and 4°C for 10 minutes, reduced to 0.5 to 1 mL, and stored at 4°C before the flow cytometry and FACS analyses.

Flow Cytometry/FACS

Flow cytometry examination was carried out on a FACS calibur (Serial number E1444, Becton-Dickinson Immunocytometry Systems, 2350 Qume Drive, San Jose, Ca). Instrument settings for *C. parvum oocyst* and *G. lamblia* cysts were listed in Table 2.1.

A standard sample data acquisition template was used to determine the range of FSC (Forward Scattered Light), SSC (Side scattered light) and FL1 (Fluorescent Light) values. A particle would need to fall into the ranges to be considered as a possible *C. parvum* oocyst or *G. lamblia* cysts. Region R1 (on a FSC vs. SSC plot) defined an area within which particles with size and internal complexity consistent with *C. parvum*

oocyst and *G. lamblia* cysts would be located. Region R2 on a FSC vs. FL1 plot defined an area within which particles of the size and fluorescence of fluorescein isothiocyanate-labelled (FITC labelled) *C. parvum* oocyst and *G. lamblia* cysts would be located.

Table 2. 1: Instrument settings for *C. parvum* oocyst and *G. lamblia* cysts

Instrument setting	*C. parvum*	*G. lamblia*
Forward scatter photodiode setting (linear mode)	E00 with 4.00 amp gain	E-01 with 7.04 amp gain
Side scatter photomultiplier setting (linear mode)	402 with 4.00 amp gain	350 with 4.00 amp gain
Fl1 photomultiplier setting (log mode)	470 with 1.00 amp gain	480 with 1.00 amp gain
Fl2 photomultiplier setting (log mode)	578 with 1.00 amp gain	578 with 1.00 amp gain
Threshold primary parameter	FSC at a value of 52	
Compensation	FL1-1.3%FL2	

Sorted samples were passed through a transparent cell culture insert (12 well format, 1 µm pore size, Becton Dickinson, Franklin Lakes, NJ) to retain (oo)cysts and other particles. Following sorting, the membrane was cut out of the holder and placed on a glass slide. Seven µL water was added to the membrane, and a cover glass placed on top. The cover glass was ringed with nail polish. The membrane was examined using an epifluorescence microscope at 400x magnification. *C. parvum* ocyst and *G. lamblia* cysts were identified and counted.

2.1.4 G. lamblia Infectivity Assay

Due to the difficulties that were encountered during the study, the procedure for purification of the parasites from the wastewater concentrates by IMS was eventually replaced with sucrose flotation. Likewise, the enumeration of the parasites by flow cytometry/FACS was replaced by epifluoresence microscopy. These modified procedures

were used in a set of wastewater analysis experiments in which the objective was to determine the infectivity of *G. lamblia* cysts (chapter 4) using the Mongolian gerbil model. Gerbils were inoculated with wastewater concentrates that were purified using sucrose flotation. The presence of infection in the gerbils was based on either presence of cysts in the feces or trophozoites in the intestine.

The eluted sample was transferred into several 50 mL centrifuge tubes and was centrifuged at 388×g for 10 minutes. Following centrifugation, the supernatant was aspirated down to 1 mL. The 1 mL concentrate was carefully overlaid on 3 mL of 1M sucrose in a 15 mL centrifuge tube. Tubes were then centrifuged for 15 minutes at 285×g. Following centrifugation, the *G. lamblia* cysts layer was directly above the sucrose layer; the top layer was therefore removed and discarded by pipette and the subsequent "milky" layer containing the cysts was pipetted into another 15 mL centrifuge tube. The tube was filled with double de-ionized water produced from a Milli-Q water purification system (hereafter referred to as Milli-Q water) and the contents in the tube were centrifuged at 388×g at 4°C for 10 minutes to remove residual sucrose and lighter debris. The final sample volume was made up to 3 mL with Milli-Q deionized water. The purified samples were gently pipetted up and down and kept in 4°C refrigerator.

Inoculation

About 0.2 mL of the final concentrated and purified samples from both upstream and downstream of the UV were inoculated to Mongolian gerbils. The gerbil inoculations were done intragastrically using a blunt needle attached to the end of a syringe. The needle was inserted through the esophagus into the animal's stomach. These 4 to 8 weeks old male Mongolian gerbils were ordered form Charles River company and shipped from

Kingston (NY.) to University of Alberta. They were housed in filter top cages and fed with commercial lab chow (#501 rodent diet) plus sunflower seeds. Gerbils' cages were changed once a week to keep them clean. Gerbils were maintained at the Biological Sciences Animal Care Facility at University of Alberta, and the room temperature was set between 68°F to 70°F. Typically, ten gerbils were inoculated with the same wastewater concentrate sample and the number of gerbils that scored positive for infection was recorded.

Cyst Detection

The methodology for cyst detection in Mongolian gerbil feces was adapted from Belosevic et al. (1983). Feces were collected for three sequential days between 10 and 20 days (post inoculation) prior to examination of intestines.

The Mongolian gerbils were placed on false-bottom collection cages, according to sample number and earmarks. A small amount of water was employed in the cages to soften the feces. About 1.5 mL Milli-Q water was dispensed into 14 mL tubes and the tubes were then weighed. After approximately 1 hour, the feces were collected in pre-weighted tubes and the tubes were re-weighed to determine the mass of wet feces.

The contents of each tube were emulsified and gently pipetted onto 3 mL of 1 M sucrose in glass test tubes. The tubes were then centrifuged for 15 minutes at 285×g and 4°C. Following centrifugation, the *G. lamblia* cysts layer was directly above the sucrose layer. The top layer was removed and discarded by pipette and the subsequent "milky" layer containing the cysts was pipetted through 40 µm filters resting on 15 mL centrifuge tubes. The tubes were filled to the top with Milli-Q water and centrifuged for 10 minutes

at 388×g and 4°C. The supernatant was removed to 1 mL and the pellet was re-suspended by gently pipetting the solution up and down. Feces were examined by microscopy for the presence or absence of cysts. Four complete sides of a haemocytometer slide were examined for each feces sample.

Trophozoite Detection

The methodology for trophozoite detection in Mongolian gerbil intestines was adapted from Belosevic et al. (1983). The Mongolian gerbils were terminated by cervical dislocation 13 days after inoculation. The entire small intestine was removed and sectioned into four pieces: section 1 being closest to the stomach and section 4 being closest to the caecum. Each segment was placed in 3 mL PBS in a 15 mL centrifuge tube and the tubes were placed on ice for 30 minutes.

The intestinal segments were removed from the tubes, were cut longitudinally and the mucosal layer was scraped off. Both the mucosal layer scraped off from the intestine and the intestinal segment itself were returned to the centrifuge tube. The tube was placed on ice for another 30 minutes. The suspension in the tube was vortexed and the contents were strained through gauze into another 15 mL centrifuge tube containing 3 mL PBS. The tube was filled with PBS to 8mL total volume and was centrifuged for 15 minutes at 285×g and 4°C. The volume was reduced to 1mL. Two haemocytometer counts were performed on the suspensions resulting from segments 1 and 2 and one count was performed on fragments 3 and 4.

2.2 Parasite Production and Purification

2.2.1 *C. parvum*

The *C. parvum* strain used for recovery trials was commonly referred to as the Iowa strain. The oocyst strain was originally isolated from the Iowa strain and has been maintained in University of Alberta laboratories for several years. Oocysts were isolated from the feces of infected neonatal Holstein calves according to Belosevic et al. (2000) via sieves followed by cesium chloride (CsCl) density gradients and centrifugation. Male Holstein calves were experimentally infected four days after birth and the feces were typically collected between 4 and 10 days following infection. The feces was strained through a series of sieves (420, 150 and 75μm), was then concentrated by centrifugation (Beckman L8-70M Ultracentrifuge, Rotor type SW28, 11,000 RPM), and the supernatant was removed. The fecal pellets were then carefully layered onto discontinuous CsCl gradients.

Prior to layering the density gradients, the following stock solutions were prepared. Tris-EDTA consisted of 500 mL deionised water with 3.94 g Tris-HCl, 1.46 g EDTA and PH was adjusted to 8.0. A CsCl stock solution was made by dissolving 180 g CsCl (99.5% Cat#Em-3045, VWR) in 100 mL deionised water. The following working stocks were then prepared using the above solutions: 1.4, 1.1 and 1.05 g/mL CsCl. A discontinuous CsCl gradient was prepared in a 40mL Beckman polyallomer centrifuge tube by gently layering 9 mL of the 1.4 g/mL CsCl stock first, followed by 18 mL of the 1.1g/mL CsCl stock and 9 mL of the 1.05 g/mL CsCl stock. The calf feces was then gently layered on top of the CsCl gradient and centrifuged at 11,000rpm(Beckman L8-70M Ultracentrifuge, Rotor type SW28) for 60 minutes at 4°C. The layer which

contained the purified oocysts was removed and washed by Milli-Q water for 10 minutes at 11000 rpm (Beckman L8-70M Ultracentrifuge, Rotor type SW28) twice.

Antibiotics (100-U/ml each of penicillin, streptomycin and gentamycin) were added to the purified oocyst stock, to prevent bacterial growth. The stock was stored at 4°C. Oocysts were used for up to 90 days post isolation.

2.2.2 G. lamblia

G. lamblia cysts (W.B. strain) were produced *in vitro* by an adaptation of the protocol outlined by Belosevic et al. (1983) (Appendix 7.3). Trophozoites were stored at -80°C in a 10 percent dimethyl sulfoxide (DMSO $(CH_3)_2SO$, BDH INC. CAS 67-68-5) solution and were cultivated in Diamond's TYI-S-33 medium which consisted of trypticase-peptone 10 g, yeast extract 5.0 g, dextrose 5.0 g, sodium chloride 1.0g, L-cysteine hydrochloric acid 1.0g, ascorbic acid 0.1g, K_2HPO_4 0.5g, KH_2PO_4 0.3g, ferric ammonium citrate 0.01g, calf serum (Atlanta Biological Cat# S 11250) 50 ml, vitamin additive (NCTC-13 from Gibco-BRL) 15 ml, gentamycin (50 mg/L from Gibco-BRL) 0.5 mL and Milli-Q water up to 500 ml. The pH was adjusted to 6.8 with sodium hydroxide and the media was filtered through 0.8 (Milli-Fil- P.F., Millipore Corp.) and 0.22 µm filters (Sterivex-GS filters with filling bell, Millipore Corp.).

When the culture had grown to confluence at 37°C, the trophozoites were encysted using Diamond's TYI-S-33 medium supplemented with 10 mg/mL bovine bile and 0.5 mM lactic acid with a pH of 7.8. After 24 hours the culture was placed on ice for 10 minutes and was shaken to loosen the cysts. The contents were then centrifuged at 388×g and 4°C for 10 minutes. The supernatant was decanted, and filled with growth media to transfer cysts into a cell culture flask where they were incubated for another 24

hours at 37°C. The cysts were once again centrifuged at 388×g for 10 minutes and the supernatant was decanted. Cysts were then suspended in Milli-Q water.

Cysts that were four days old or younger were purified using CsCl density gradients. A CsCl stock solution with a specific gravity of 1.80 was prepared by adding about 360 g of CsCl to Tris-HCl EDTA buffer. The specific gravity was adjusted to 1.80 by adding more CsCl or buffer until an average weight of 1mL equalled 1.8 g. A gradient consisting of three distinct layers of CsCl solution is as follows: bottom-1 mL of S.G.=1.144, middle-2 mL of S.G.= 1.072, top-1 mL of S.G.=1.048. Approximately 0.5 mL of *G. lamblia* cyst stock preparation was gently layered on top of the gradients. The gradient was centrifuged at 10,000 rpm on a Beckman Model SW55T1 Class B-H ultracentrifuge. The cyst-rich layer was carefully removed and re-suspended in Milli-Q water. The suspension was centrifuged at 388×g and 4°C for 15 minutes. The supernatant was removed and discarded. This wash step was repeated and the cyst preparation was store in Milli-Q water at 4°C.

3 EVALUATION OF PARASITE RECOVERY METHODS

3.1 Introduction

An ideal method of recovering and detecting *Cryptosporidium* spp. oocysts and *Giardia* spp. cysts from water samples would provide high and consistent recoveries as determined in recovery tests in which samples are spiked with a known number of (oo)cysts. The method would also result in few false positives or false negatives, would indicate the species of parasite and would provide indication of viability and, ideally, the potential to infect humans. An additional criterion for the wastewater study described in this thesis was that the method would permit evaluation of infectivity using established animal infectivity models. For this, the concentrated sample would need to be sufficiently purified to allow inoculation of the sample into experimental animals.

Two widely used protozoan detection methods within water industry were developed by the United States Environmental Protection Agency (USEPA). The first method is the Information Collection Rule (ICR) (USEPA, 1995) which was intended to provide USEPA with information on chemical by-products that form when disinfectants used for microbial control react with chemicals already present in source water [(disinfection by-products (DBPs)]; disease-causing micro-organisms (pathogens), including *Cryptosporidium* and *Giardia*; and engineering data to control these contaminants. The ICR method consists of filtration, elution, Percoll-sucrose flotation, an indirect immunofluorescent antibody (IFA) staining assay, and microscopic enumeration. However, the recoveries of protozoa were shown to be low and variable when samples of

water were spiked with a known number of (oo)cysts in treated water (Clancy et al. 1997, Bukhari et al. 1998).

The second method is "Method 1623: *Cryptosporidium* and *Giardia* in Water by Filtration/ IMS/ FA" (USEPA 1999). In this method a capsule filter and immunomagnetic separation (IMS) are used prior to analysis by immunofluorescence microscopy. Although this method had been shown to have higher recovery efficiency and greater precision than the ICR method, they still suffer from the limitations of an antibody-based method, principally the inability to assess the infectivity and public health significance of the detected organisms.

Although most of the basic techniques such as filtration and the immunofluorescence identification used by these two methods are similar, Method 1623 is still subject to turbidity problems but less so than the ICR method. The major improvement is the use of immunomagnetic separation. The new separation technique allows for less vulnerability to turbidity levels and a cleaner sample. Another key difference between the two methods is that method 1623 uses a direct, fluorescein-labelled, combination, dual monoclonal antibody cocktail kit to detect *G. lamblia* cysts and *C. parvum* oocysts by direct immunofluorescence; while the method ICR uses indirect, fluorescein-labelled, monoclonal antibody-based kit to detect *Giardia cysts* and *Cryptosporidium oocysts* by indirect immunofluorescence.

The method used in this thesis was adapted from that in Michener's M.Sc. thesis (Michener, 2002). Essentially, Michener's method is a modification of Method 1623. Michener employed species specific antibodies for IMS and parasites detection. The use of flow cytometry detection in Michener's method resulted in the reduction of the

variability, time and effor. The use of infectivity analysis in animal models made the viability determination of *C. parvum* and *G. lamblia* successful.

All the aforementioned methods were developed primarily for treated and untreated drinking water. Treated municipal wastewater is generally much higher in suspended solids and dissolved organic content compared to treated or untreated drinking water. The goal of this project was to find out if these methods also work well for treated municipal wastewater.

3.2 Experimental Objectives

The objectives of the experiments described in this chapter were to evaluate the Michener method for recovering and enumeration cysts and oocysts present in treated municipal wastewater. In the first set of experiments, the recovery of prepared *C. parvum* oocysts spiked into deionized water was measured using the Michener method. In the second set of experiments, the recovery of prepared and immunofluorescently-labelled *G. lamblia* cysts was measured using a modification of the Michener method in which the Miltenyi Biotech IMS purification procedure was replaced with sucrose flotation.

3.3 Cryptosporidium parvum Recovery Experiments Using Michener Sampling and Analysis Procedure

3.3.1 Procedure

A 10 L carboy was filled with deionized (DI) water to approximately 5 L. This was spiked with a known number of prepared *C. parvum* oocysts, counted using haemocytometer. Continuous mixing of the carboy contents was done using a magnetic

stirrer. The water was pumped from the carboy through a clean Filta-Max™ filter using the sampling apparatus described in Chapter 2 (Figure 2.3). The carboy was continuously filled with fresh deionized water and pumping was continued until a total of 50 L of water had passed through the filter. Following filtration, the material concentrated on the filter was eluted according to the procedure described in Chapter 2. A membrane filter was employed to concentrate eluted sample into a pellet. The pellet was then purified using the Miltenyi Biotech IMS system and oocysts were detected by flow cytometry.

The experimental flowchart is described in Figure 3.1.

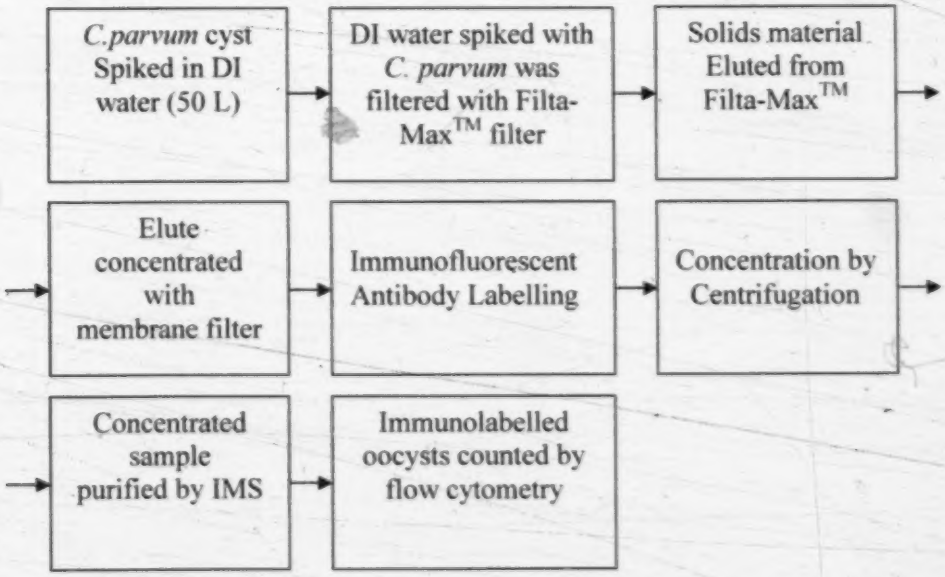

C.parvum cyst Spiked in DI water (50 L)
DI water spiked with C. parvum was filtered with Filta-Max™ filter
Solids material Eluted from Filta-Max™
Elute concentrated with membrane filter
Immunofluorescent Antibody Labelling
Concentration by Centrifugation
Concentrated sample purified by IMS
Immunolabelled oocysts counted by flow cytometry

Figure 3. 1: Procedural flow diagram for *C. parvum* recovery experiments

3.3.2 Results

The first series of *C. parvum* oocyst recovery tests were done with 50 L DI water spiked with either 500 or 10,000 *C. parvum* oocysts. In the spiked experiment, a set of 500 oocysts were spiked into 50 L DI water, which resulted in a oocyst concentration of

10/L (500/50L = 10/L). This oocyst concentration was comparable to that of very contaminated drinking water. The recovery rate for 500 oocysts was expected to be similar to what Michener obtained for untreated drinking water. The oocysts concentration in wastewater ranges from 3.3 to 20,000/L (Smith, 1990) which is much higher than that found in drinking water. It was important to do the recovery experiment using 10,000 oocysts because the resulting concentration is 200/L (or 10,000/50L = 20,000/100L) which falls into the range of oocysts concentration in wastewater (Smith, 1990). The results of these experiments are summarized in Table 3.1.

Table 3. 1: Recovery experiment results for 50 L DI water spiked with *C. parvum* oocysts (10,000 and 500)

Number of oocysts spiked	Number of oocysts detected	Recovery %	Mean of trials %	Standard Deviation %	Coefficient of Variation
10,000	888	8.88	12.7	5.4	42.5
10,000	1645	16.45			
500	273	54.6	61.5	9.8	15.9
500	342	68.4			

The recovery using 500 oocysts was relatively good and the result were similar to those reported by Michener (2002). The recovery using 10,000 oocysts was much lower. The reason for such a large difference in recovery rates observed between the two oocysts concentrations was probably due to oocysts clumping. Microscopic examination of the concentrated samples, following IMS purification, revealed the presence of small clumps composed of two or more oocysts. Oocysts identification by flow cytometry was based on size, the instrument was unable to distinguish single versus multiple oocysts. A second set of experiments was carried out to investigate factors that might lead to formation of

oocysts clumps. In these experiments, 20,000 prepared oocysts were mixed with various reagents as described in Table 3.2. The samples were observed using light microscopy and a haemocytometer[2]. The number of clumps and number of oocysts per clump were observed and recorded. Results are provided in Table 3.2.

Table 3. 2: Clumps of *C. parvum* oocysts observed in different reagents

Number of oocysts Spiked	Composition of Reagent Mixture	Clumps	
		Number of Clumps Observed	Number of oocysts per clump
20,000	9.5 mL IMS buffer	No clump observed	No clump observed
20,000	1%Tween 80 1 mL, 8.5 mL IMS buffer	No clump observed	No clump observed
20,000	10 µL anti-*Cryptosporidium* Antibody (ImmuCell), 9.5 mL IMS buffer	2	2
20,000	10 µL anti-*Cryptosporidium* Antibody (ImmuCell), 8.5 mL IMS buffer, 1 mL 1%Tween 80	2	2
		1	3
20,000	10 µL anti-*Cryptosporidium* Antibody (ImmuCell), 9.5 mL IMS buffer, 20 µL microbeads	1	2
		1	5
		1	25
20,000	10 µL anti-*Cryptosporidium* Antibody (ImmuCell), 9.5 mL IMS buffer, 20 µL microbeads, 1 mL 1%Tween 80	1	5

The clump detection tests were done using the complete IMS procedure described in Chapter 2. Number of oocysts per clump and number of clumps were detected under Nikon Microphot-FXA microscope with 20× magnification using haemocytometer. No clumps were observed when *C. parvum* oocyst were incubated with IMS buffer and 1%

[2] Nikon Microphot-FXA microscope filters are ND2, ND8, ND32 used for general microscopy and brightness adjustment in photomicrography, filter HE is color photomicrography, filter GIF is green interface Filter, filter NCB 11 is color balance filter. Hausser Scientific.Brightline Hemacytometer 0.100 mm deep

Tween 80 solution only. Similar size and frequency of clumps were observed when *C. parvum* oocysts were incubated with other reagents. Most of the clumps observed in the experiment consisted of two *C. parvum* oocysts. Larger sizes of clumps (consisting more than five *C. parvum* oocysts) were also observed.

According to Table 3.2, addition of the antibody seemed to be the key variable in formation of clumps. Addition of microbeads may have contributed further to clump formation. Addition of Tween 80 did not prevent formation of clumps.

Each antibody consists of four polypeptides—two heavy chains and two light chains joined to form a "Y" shaped molecule (Figure 3.2). It is possible for one antibody to bind with two antigens because of the existence of two antigen binding sites on each antibody, therefore, formation of clumps could not be avoided when more than one *C. parvum* oocyst conjugated with one anti-*Cryptosporidium* antibody.

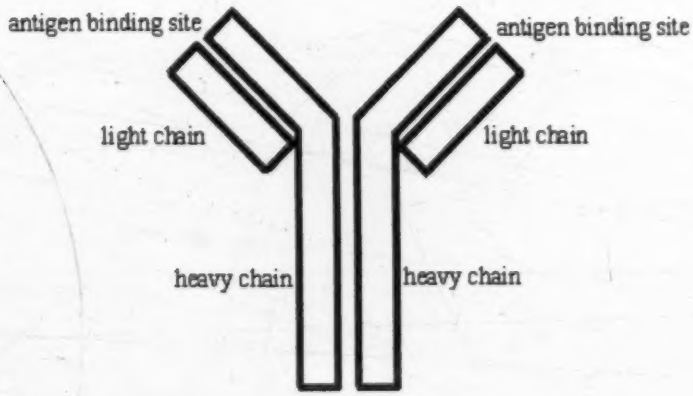


Figure 3. 2: Structure of antibody

Centrifugation may also contribute to clump formation. When centrifugation was used to concentrate water sample, most of the *C. parvum* oocysts were concentrated at the bottom of centrifuge tube. Therefore, when concentration of oocysts was increased, formation of clumps increased. This accounts for the poorer recovery at higher oocyst concentration demonstrated in Table 3.1. Ultimately it means that the method used by Michener might not be suitable for a wastewater application where the parasite concentration might be higher.

3.4 Giardia lamblia Recovery Experiments Using Modified Sampling Procedure

3.4.1 Procedure

Freshly prepared *G. lamblia* cysts were labelled with FITC conjugated antibody using the procedure described in Section 2.1.3. Because the filtration for *G. lamblia* spike tests was done at the Goldbar wastewater treatment plant, it was necessary that no live *G. lamblia* cyst were present. Prior to use in spike tests, the cysts were killed by heating to 70°C for 20 minutes. After being killed by heating, no distinct fluorescence decrease of stained *G. lamblia* cysts was detected before use in experiments. A preparation of antibody labelled heat inactivated cysts was enumerated by flow cytometry and then pipetted onto the upstream surface of the Filta-max filter. The filter was then sealed in a filter housing in preparation for either filtration of wastewater at Edmonton Goldbar WWTP or filtration of deionized water at University of Alberta laboratories. Following filtration, the material concentrated on the filter was eluted according to the procedure described in Section 2.1.2. A membrane filter was employed to concentrate elution

samples into a pellet. The background material in the pellet was then reduced by discontinuous density gradient centrifugation using 1.0 M sucrose. Centrifugation caused denser particles to pass through the density medium and form a pellet at the bottom of the tube. Theoretically, live *Giardia* cysts would have floated on the surface of the density medium and been recovered by pipette. Dead cysts tend to penetrate the density flotation medium and accumulate in the pellet (Bukhari and Smith 1996). The recovered material was then centrifuged and concentrated again, and the final pellet was examined by epifluorescence microscopy. The experimental flowchart is described in Figure 3.3

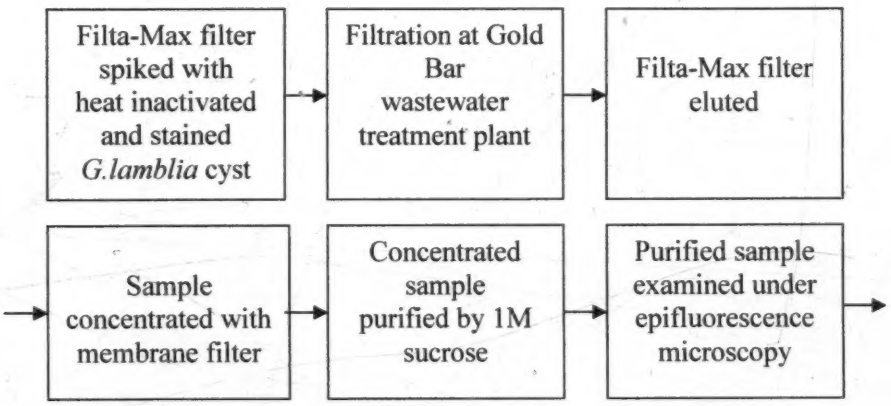

Filta-Max filter spiked with heat inactivated and stained *G.lamblia* cyst
Filtration at Gold Bar wastewater treatment plant
Filta-Max filter eluted
Sample concentrated with membrane filter
Concentrated sample purified by 1M sucrose
Purified sample examined under epifluorescence microscopy

Figure 3. 3: Procedural flow diagram for *G. lamblia* recovery experiments

3.4.2 Results

Recovery tests for *G. lamblia* were carried out on four filtration samples A, B, C and D. Sample A and B were collected from Gold Bar wastewater treatment plant upstream of the UV reactor. Samples C and D were processed with deionized water at

University of Alberta laboratories. The details and the results of the *G. lamblia* recovery experiments are summarized in Table 3.3.

Table 3. 3: Recovery test results for *G. lamblia* spiked into secondary wastewater and deionized water

	Wastewater		**DI water**	
	Sample A	Sample B	Sample C	Sample D
Number of *G. lamblia* Spiked[1]	35000±6650	35000±6650	35000±6650	35000±6650
Volume of water processed (L)	150	150	150	150
Volume of concentrates (mL)	0.630	0.575	0.668	0.716
Number of *G.lamblia* After purification (detected by microscope)[2]	1575±347	2516±604	5845±994	6265±1253
Recovery %	4.5	7.19	16.7	17.9
Mean Recovery%	5.85		17.3	

1: Samples spiked with 300µL *G. lamblia* cysts from the same *G. lamblia* stock solution. Number of *G.lamblia* cysts is based on four haemocytometer counts of the stock solution.
2: Number of *G.lamblia* cysts is based on four haemocytometer counts of recovered samples.

As illustrated in Table 3.3, the number of *G. lamblia* detected after purification was lower in wastewater samples (A and B) than in DI water samples (C and D). Recoveries of *Giardia* cysts were low and variable. The average percent recoveries for *G. lamblia* spiked in wastewater and deionized water was 5.85% and 17.3% (Table 3.3), respectively. The difference between the recovery rates of *G. lamblia* in wastewater and DI water may be due to the different matrices and chemical characters of wastewater and DI water. The wastewater used in the recovery trials was high in suspended solids, and some cysts may attach to these particles. The attached cysts tend to end up at the bottom of the sucrose tube and will not be recovered.

Further research on the selection of new antibody, efficient separation system and optimizing current protozoan detection assay should be conducted to examine whether a higher recovery rate with smaller coefficients of variation can be attained.

The recovery experiments that were conducted predicted what fraction of the cysts naturally present in the wastewater were present after filtration and sucrose purification. They did not predict the ability of the IFA procedure for enumeration of cysts following filtration and sucrose purification. Therefore, the recoveries obtained in these experiments do not necessarily represent the actual recoveries of *G. lamblia* at Goldbar WWTP and the WWTP in City of Kelowna. In Chapter 4, the Mongolian gerbil model was used to determine the infectivity of *G. lamblia* cysts found in secondary wastewater. The recovery experiments reported here are an indication as to know how many of the cysts present in the wastewater actually were inoculated to each gerbil.

3.5 Summary

The low recovery rates of oocysts in wastewater samples suggest that Michener's method is not suitable for concentration and detection parasites in wastewater. Two major reasons are as follows:

1. The direct antibody used for *C. parvum* may result in clumping of oocysts at the higher concentrations that are likely to be encountered in wastewater. This confounds flow cytometry detection.
2. The Miltenyi Biotech IMS system is unsuitable for purification of turbid and large volume of wastewater concentrates as the separation columns become plugged with wastewater solids.

A modified procedure in which IMS was replaced by sucrose flotation recovered about 6% if the cysts present in wastewater. This may be sufficient to carry out infectivity analyses. For example, a previous study showed that cysts concentration in the Goldbar effluent was between 8,100/100 L and 56,000/100 L (Shepel 2000). If 300 L wastewater was sampled with a 6% recovery, and assuming a 50% recovery for the Shepel data, then the number of cysts recovered would range between 1,500 and 11,000. If the inoculum is divided between 10 gerbils, this would result in inoculum of between 150 and 1,100 per gerbil. Given that the ID_{50} for the Mongolian gerbil model has been estimated to be about 250 (Rendtorff, 1978; Nash et al., 1987), the recovery should be sufficient to measure infectivity of cysts present in secondary wastewater.

4 INFECTIVITY OF *G. LAMBLIA* CYSTS IN WASTEWATER AND EFFECT OF UV TREATMENT

4.1 Introduction

In this chapter, the Mongolian gerbil model was used to determine the infectivity of *G. lamblia* cysts in UV-treated wastewater effluents at two municipal wastewater treatment plants, Goldbar WWTP in City of Edmonton and the WWTP in City of Kelowna.

A series of field experiments are described in this chapter in which the infectivity of *G. lamblia* cysts recovered from secondary wastewater effluent at the WWTP in City of Kelowna and Goldbar WWTP in City of Edmonton was measured. The objectives were to: (1) determine if cysts present in the secondary effluent are infectious, and (2) determine to what degree the infectivity is reduced in field UV reactors. At both study sites, the infectivity was measured for samples collected immediately upstream and immediately downstream of operating UV reactors on the same day. Samples were collected and concentrated using the Filta-Max™ filtration procedure and shipped to the laboratories at University of Alberta. The concentrates were eluted from the filter and were purified using the sucrose procedure that was tested in Chapter 3. Infectivity of the purified concentrates was then determined by using the Mongolian gerbil model (Belosevic et al., 1983). It was important to use the gerbil model for determining infectivity because *in vitro* surrogate methods, such as *in vitro* excystation or vital dye assays do not provide an accurate indication of the effect of UV on the infectivity of the

parasites (Craik et al., 2001). Sub samples were also enumerated using the immunofluorescence assay with both flow cytometry and microscopic detection.

4.2 Experimental Conditions

About 0.2 mL of the final concentrated and purified sample collected from both upstream and downstream of the UV reactors was inoculated orally to each Mongolian gerbil to test the infectivity of *G. lamblia*. Infective *G. lamblia* cysts were detected by checking Mongolian gerbils' feces 10 days after inoculation. After checking the feces for 3 days, the animals were sacrificed, dissected and the intestines were checked for the presence of trophozoites (Chapter 2). Three experiments were conducted at each study site on different days during the months June, July and August of 2003. The chronology of these experiments is described in Table 4.1 and Table 4.2 for City of Edmonton and City of Kelowna, respectively. These tables indicate the actual dates of field sampling, gerbil inoculation, feces and intestinal checks. The volume of filtered secondary wastewater from both cities is summarized in Table 4.3.

To test the potential of cross-contamination between gerbils and of gerbils being infected before they were inoculated using concentrated and purified wastewater samples, a few negative control animals were employed. In the negative control, gerbils received only normal commercial lab chow, sunflower seeds and water. In addition, the positive controls in which inoculated gerbils with freshly prepared *Giardia* cysts was conducted. The positive controls ensured that the gerbils used in the studies were capable of being infected should there be a certain number of *Giardia* cysts existing in the concentrated and purified wastewater samples.

Table 4. 1: Summary of wastewater infectivity analysis and experiment date for City of Edmonton samples

Set of experiment		#1	#2	#3
Date (2003)	Filtration	Jun.10	Aug.12	Aug.26
	Inoculation	Jun.13	Aug.15	Aug.29
	Feces check	Jun.23, 24, 25	Aug.25,26, 27	Sep. 8, 9, 10
	Intestinal check	Jun.26, 27	Aug.28	Aug.11
Number of gerbils used	Total	20	16	22
	Upstream UV reactor	10	6	10
	Downstream UV reactor	10	6	8
	Positive control	0	2	0
	Negative control	0	2	4

Table 4. 2: Summary of wastewater infectivity analysis and experiment date for City of Kelowna samples

Set of experiment		#1	#2	#3
Date (2003)	Filtration	Jun. 21	Jul. 21	Aug. 18
	Inoculation	Jun. 24	Jul. 25	Aug. 22
	Feces check	Jul. 7, 8	Sep. 5, 6,	Sep. 2, 3, 4
	Intestinal check	Jul. 9, 10	Aug. 6, 7	Sep. 4
Number of gerbils used	Total	20	20	14
	Upstream UV reactor	10	10	6
	Downstream UV reactor	10	10	6
	Positive control	0	0	0
	Negative control	0	0	2

Table 4. 3: Summary of filtered secondary wastewater volume for City of Edmonton and City of Kelowna

	SET #	FILTRATION VOLUME	
		DOWNSTREAM (L)	UPSTREAM (L)
City of Edmonton	1	300	300
	2	300	300
	3	300	300
City of Kelowna	1	1220	1257
	2	1166	1202
	3	1393	1245

General information regarding the WWTPs in City of Edmonton and City of Kelowna and the average treated wastewater characteristics was provided by engineers from these two study sites and is summarized in Table 4.4 (Detailed information is provided in Table 7.23 to Table 7.40 in the Appendix).

Table 4. 4: General information of treated wastewater effluent from City of Edmonton and City of Kelowna

	City of Edmonton	City of Kelowna
Total Suspended Solid (mg/L)	3 to 5	<1
Calculated UV dose mJ/cm^2	30	50
%Transmittance (254nm)	61 to 65	60
Model of UV reactor	Trojan 4000	Trojan 3000

The suspended solid concentration in City of Kelowna wastewater was much lower than that in City of Edmonton. This was due to the teriary sand filtration practiced in City of Kelowna. The comparatively lower suspended solid concentration suggested it

was necessary to collect samples of greater volume in City of Kelowna than in City of Edmonton.

Both WWTPs employed UV disinfection systems, however, the model of the UV reactors and the UV dose was different. The UV dose during the test period was 50 mJ/cm^2 at City of Kelowna and 30 mJ/cm^2 at City of Edmonton, respectively. For a given UV reactor configuration, the delivered dose is a function of the output of the individual lamps, the transmittance of the water and the exposure time. In the Trojan UV 3000 and UV 4000 reactors, the delivered dose is calculated using a computer program that accounts for these factors. The output of the lamp and the transmittance of the water are accounted for by measuring the UV intensity at the wall of the UV reactor channel. The exposure time is determined by monitoring the volumetric flow rate of the water. It is important to note that the calculated dose is an average dose. In reality, micro-organisms passing through the reactor receive different doses resulting a dose distribution. In addition, the calculated dose does not account for the effect of particulate matter. For example, a *Giardia* cyst that is embedded within a wastewater floc particle may receive a much lower UV dose than the calculated average dose.

4.3 Level of Infectivity

At the outset, the expected infection result for each gerbil was assumed to be either positive or negative. However, the initial observations indicated that there were different levels of infection. Therefore, an arbitrary infection scale was developed to capture these differences. The degree of infection observed in the gerbils of infectivity was divided into four different levels based on results from checking for cysts in the gerbil feces and checking for trophozoites in the intestines (Table 4.5).

Table 4. 5: The four infectivity levels

	Level	Description
-	No infection	No cysts observed in feces check; No trophozoites detected in intestines
+	Low infection	Cysts may or may not be observed in feces check; Trophozoites were detected with slight flagella movement or no movement (dead trophozoites)
++	Moderate infection	Cysts may or may not be observed in feces check; Trophozoites were detected with distinct flagella movement
+++	Heavy infection	Cysts are observed in feces check; A large number of trophozoites were detected and exhibited a distinctive erratic twisting motion, (comparable to a falling leaf)

4.4 Results

Purified wastewater samples collected from both upstream and downstream of the UV reactors at both WWTPs were divided into two identical sets. One set was used for enumeration and the other was used for infectivity tests using gerbils.

The results of the enumeration and infectivity tests using the gerbil model are described below.

4.4.1 Results for Giardia lamblia Detection

Both flow cytometry and epifluorescent microscopy were used for enumerating *G. lamblia* cysts in the sucrose purified wastewater concentrates for all samples collected in City of Edmonton and City of Kelowna.

The enumeration tests were done before gerbils were infected. A 0.3 mL sub-sample of wastewater that was purified using 1M sucrose was conjugated with anti-*Giardia* antibody and anti-mouse antibody (Chapter 2), followed by flow cytometry detection and epifluorescent microscope detection. For all the wastewater concentrates collected in the two WWTPs, no *G. lamblia* cysts were detected by flow cytometry. A cluster of several *G. l amblia* cysts was observed under fluorescent microscope in a purified wastewater concentrate collected at City of Edmonton and filtered on August 26, 2003 (set #3). No such clusters were observed in other sucrose purified wastewater concentrates collected at the two study sites. The microscopy photographs taken by using conventional bright field microscopy (Figure 4.1) and UV fluorescence microscopy (Figure 4.2) show the cluster of *G. lamblia* cysts observed. Figure 4.1 shows the size and shape of clumped *G. lamblia* cysts. Figure 4.2 shows the fluorescence of *G. lamblia* cysts which conjugated with anti-*Giardia* antibody and anti-mouse. These photographs were taken under Nikon Microphot-FXA fluorescent microscope with a magnification of 20×.

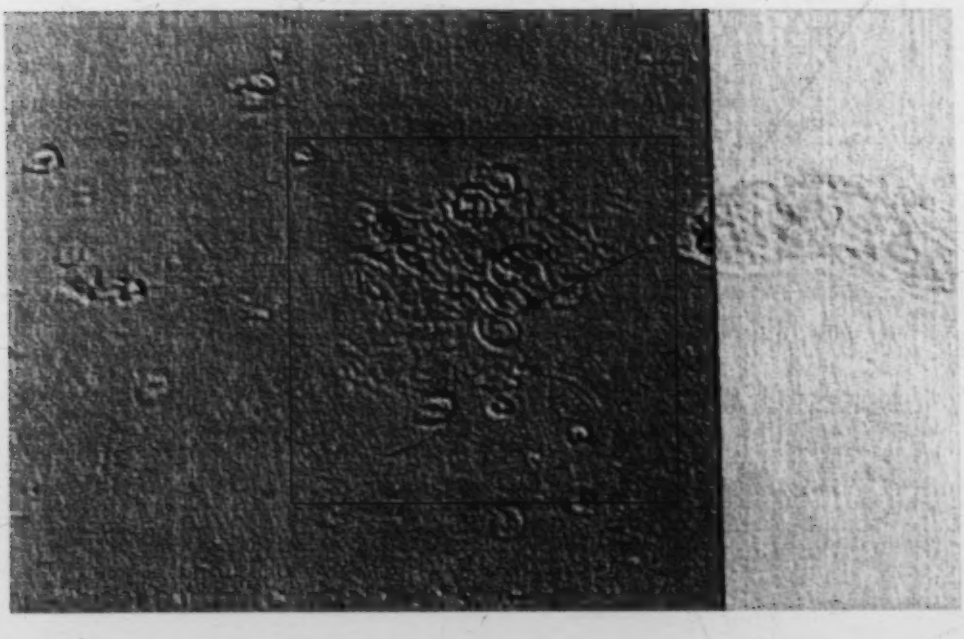


Figure 4. 1: *G. lamblia* cysts observed under conventional light microscopy in the sample of purified wastewater concentrate from City of Edmonton Goldbar WWTP(Aug. 26, 2003)

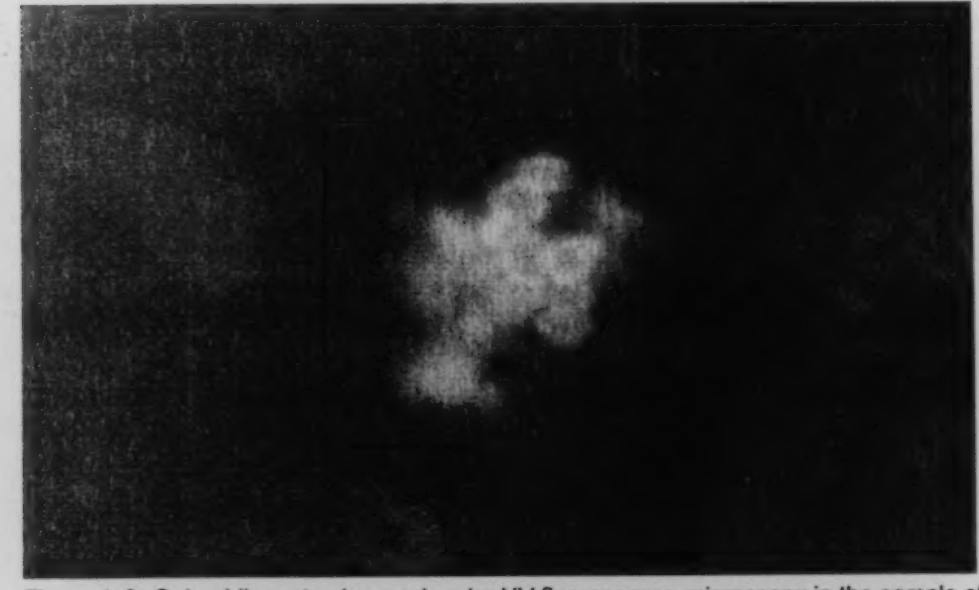


Figure 4. 2: *G. lamblia* cysts observed under UV fluorescence microscopy in the sample of purified wastewater concentrate from City of Edmonton Goldbar WWTP(Aug. 26, 2003)

No *G. lamblia* cysts were detected using flow cytometry, though the microscope photographs (Figure 4.1 and 4.2) demonstrated the likely presence of *G. lamblia* cysts in the secondary wastewater sample filtered from City of Edmonton. The sorting gate of flow cytometry was set for detecting single *G. lamblia* cysts only. Therefore, a cluster of this size (Figure 4.1) would be too large to be captured by the defined sorting gate. If cysts were present primarity as clusters in the secondary wastewater, flow cytometry would not be able to enumerate the existing cysts in the purified wastewater concentrates.

The reason that *G. lamblia* cysts clumped in wastewater remains unclear. One of the possible explanations for the clustering of *G. lamblia* is that the cysts in wastewater may be of different age. Yakub et al. (2000) reported that aged *G. lamblia* cysts tended to clump easier than fresh *G. lamblia* cysts. In their spike tests, Yakub and his co-workers did not observe clumps when fresh prepared *G. lamblia* cysts were used. The clustering could also simply be an artefact of the sampling and concentration procedure employed in this study. In a previous study of *G. lamblia* detection at Edmonton Goldbar WWTP (Shepel, 2000), no such clustering was reported.

Similar to particles that cause turbidity, microorganism clumps can impact disinfection efficiency by harbouring pathogens within the clumps and shading pathogens that would otherwise be inactivated. The significance of the impact of the clustering on the efficiency of inactivation needs to be further studied.

4.4.2 Infectivity Testing Results

City of Edmonton

Three sets of wastewater sampling were conducted at the City of Edmonton during the summer of 2003 (Table 4.1). Samples of treated wastewater were collected

from both upstream and downstream of the UV reactors using Filta-Max™ filters. These samples were then inoculated into Mongolian gerbils to test the presence of infectious *G. lamblia* cysts in the wastewater. For each gerbil in each sample set, the degree of infection observed was recorded in terms of the levels of infectivity defined in Table 4.5.

Table 4.6 (Data summarized from Table 7.5 to Table 7.8 in Appendix) summarizes the infectivity testing results from sample set #2 collected at City of Edmonton. Similar infectivity testing results from sample set #1 and #3 collected from City of Edmonton are included in the Appendix (Table 7.1 to Table 7.4 and Table 7.9 to Table 7.12).

Table 4. 6: Results of infectivity testing carried out on sample set #2 collected at City of Edmonton

Cage#	Sample Source	Gerbil Number	Trophozoites observation	Feces check			Infectivity level
				Day 1	Day 2	Day 3	
1	Downstream UV reactor	1R	-	-	-	-	-
		1N	-	-	-	-	-
2	Upstream UV reactor	2R	-	-	-	Yes*	-
		2N	-	-	-	-	-
3	Downstream UV reactor	3R	-	-	-	-	-
		3N	Yes	-	Yes	-	+
4	Upstream UV reactor	4R	Yes	-	-	-	++
		4N	Yes	-	-	-	++
5	Downstream UV reactor	5R	Yes	-	-	-	+
		5N	Yes	-	-	-	+
6	Upstream UV reactor	6R	Yes	-	-	-	++
		6N	Yes	-	-	-	++
7	Positive control	7R	-	-	-	-	-
		7N	Yes	-	-	-	++
8	Negative control	8R	-	-	-	-	-
		8N	-	-	-	-	-

1. "-", no infection; "+", low infection; "++", moderate infection
2. Gerbil's number relates to cage number and gerbil's ear mark
3. R: ear marked on right ear, N: no ear mark
4. Yes*, similar shape as *G. lamblia* cyst, but smaller in size and had a glossy outer layer

Four gerbils in cage #4 and #6 inoculated with wastewater concentrates collected from upstream of the UV reactor were observed as moderately infected based on presence of live trophozoites in the intestine checking. Three gerbils that were inoculated with wastewater concentrates collected from downstream were observed as slowly infected. Two of them were from cage #5 and the other one was from cage #3. Cysts were detected on day 2 and day 3 during feces checking from gerbils 3N and 2R (gerbils were indexed by the cage number and their ear mark). No infection was detected in gerbils that were selected as negative control. One of the gerbils selected for positive control was detected as moderately infected. Infectivity test results from all three sample sets collected from City of Edmonton are summarized in Table 4.7 (Data summarized from Table 7.1 to Table 7.12 in Appendix).

There were differences in resulting infection rates between sample sets. In sample set #1 and #3, none of the gerbils inoculated with wastewater samples collected from downstream of the UV reactor was positive for infection. However, in sample set #2, 50% of gerbils scored positive for low infection. Around 80% of gerbils in sample set #1 scored positive for moderate infection. This figure decreased to 70% for sample set #2 and decreased to 30% for sample set # 3. Overall, gerbils inoculated with wastewater concentrates collected from upstream of the UV reactor seemed more likely to be infected and more likely to be heavily infected than those inoculated with wastewater concentrates collected from downstream of the UV reactor.

Table 4. 7: Summary of infectivity test in City of Edmonton

Set #	Sample	Total Number of Gerbils	Infectivity Level			
			-	+	++	+++
1	Downstream of UV Reactor	10	10	0	0	0
	Upstream of UV Reactor	10 (1 died before inoculation)	2	0	7	0
2	Negative control	2	2	0	0	0
	Positive control	2	1	0	1	0
	Downstream of UV Reactor	6	3	3	0	0
	Upstream of UV Reactor	6	2	0	4	0
3	Negative control	4	4	0	0	0
	Downstream of UV Reactor	8	8	0	0	0
	Upstream of UV Reactor	10	7	0	3	0
Tota l	Negative control	6	6	0	0	0
	Positive control	2	1	0	1	0
	Downstream of UV Reactor	24	21	3	0	0
	Upstream of UV Reactor	26 (1 died before inoculation)	11	0	14	0

1. "-", no infection; "+", low infection; "++", moderate infection, "+++", heavy infection

To further examine the significance of the impact of sample location on the infection of gerbils, a logistic regression model was employed to analyze the data in Table 4.7. The response variable, the severity of infection in gerbils, was classified into three ordinary response categories, 1=no infection, 2=low infection, and 3=moderate infection. A common slopes cumulative logit model was attempted without success because the assumption of *proportional odds* were not satisfied. Therefore, categories 2 and 3 were combined and the response variable became dichotomous, 1= no infection, 0 = low or moderate infection. A logit model was then fites to obtain the odds ratio of low

or moderate infection for those gerbils inoculated with samples collected from upstream to those inoculated with samples collected from downstream of the UV reactor.

Table 4.8 summarizes the statistical analysis results. The sample location effect was highly statistically significant (P-value = 0.003). The location where samples were collected did have an impact on the infection observed in the gerbils. The odds ratio of low or moderate infection was 8.909, with a confidence interval excluding 1. This odds ratio indicated that the gerbils inoculated with wastewater concentrates collected from upstream of the UV reactors were around 8.9 times more likely to be infected than those inoculated with concentrates collected from downstream.

Table 4. 8: Results from logistic regression analyses

Type III Analysis of Effects			
Effect	DF	Chi-Square	Pr > ChiSq
Sample location	1	8.8043	0.0030
Odds Ratio Estimates			
Effect	Point Estimate	95% Wald Confidence Limits	
Upstream vs Downstream	8.909	(2.101, 37.778)	

City of Kelowna

Similar to the experiments conducted at City of Edmonton, three sets of wastewater samples were collected from both upstream and downstream of the UV reactors using Filta-Max™ filters at the City of Kelowna wastewater treatment facility (Table 4.2). These samples were then inoculated into Mongolian gerbils to test the

presence of infectious *G. lamblia* cysts. The degree of infection observed in the gerbils was recorded for each sample set. Details of the infectivity testing results from sample sets #1, #2 and #3 collected at City of Kelowna are included in the Appendix (Table 7.13 to Table 7.22).

Table 4. 9: Summary of infectivity test conducted for City of Kelowna

Set #	Sample	Total Number of Gerbils	Infectivity Level			
			-	+	++	+++
1	Downstream of UV Reactor	10	7	3	0	0
	Upstream of UV Reactor	10	4	0	6	0
2	Downstream of UV Reactor	10	8	2	0	0
	Upstream of UV Reactor	10	1	0	8	1
3	Negative Control	2	2	0	0	0
	Downstream of UV Reactor	6	6	0	0	0
	Upstream of UV Reactor	6	5	0	1	0
Total	Negative Control	2	2	0	0	0
	Downstream of UV Reactor	26	21	5	0	0
	Upstream of UV Reactor	26	10	0	15	1

1. "-", no infection; "+", low infection; "++", moderate infection, "+++", heavy infection

Infectivity test results from three sample sets collected from City of Kelowna were summarized in Table 4.9. There are differences in resulting infections among sample sets. Around 60% of gerbils inoculated with upstream wastewater concentrates in sample set #1 scored positive for moderate infection. This figure increased to 80% for sample set #2 and decreased to 17% for sample set #3. Moreover, signs of heavily

infection in one gerbil were observed in sample set #2 (Figure 4.3). In sample set #3, 100% of the gerbils inoculated with wastewater samples concentrates collected from downstream of the UV reactor were negative for infection. However, this figure decreased to 80% for sample set #2 and 70% for set #1, respectively. The rest of the gerbils in sample set #1 and #2 were positive for low infection. Figure 4.4 shows an example of a live trophozoite detected in the lowly infected gerbils from sample set #2. None of the two gerbils in the negative control group was positive for infection.

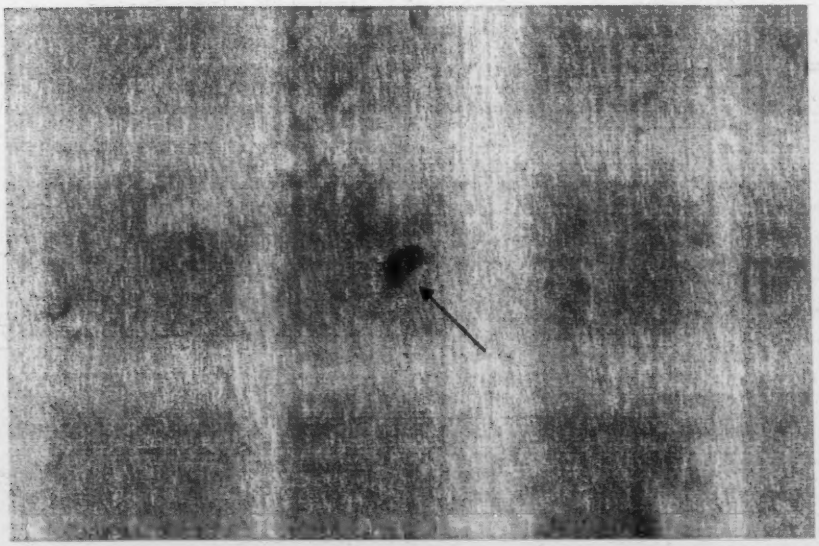


Figure 4. 3: Trophozoite detected with strong motility in intestine check from gerbil number 8R on August 7 2003-City of Kelowna set #2.

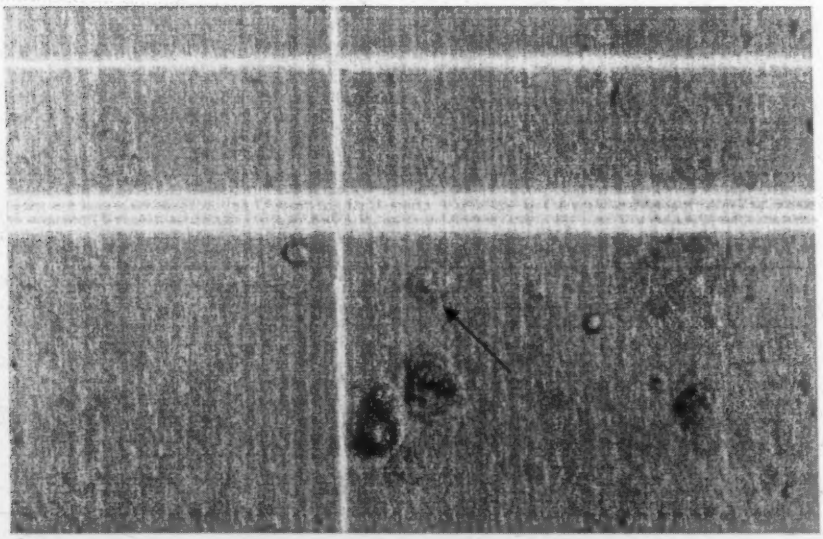


Figure 4. 4: Trophozoite detected with distinct flagella movement in intestine check from gerbil number 1N on August 7 2003-City of Kelowna set #2.

For sample set #1, no cysts were detected in the feces during the three consecutive days of checking. A large number of cysts were detected in the feces of sample set #2 on day 1 (Figure 4.5). Trophozoites exhibited a distinctive erratic twisting motion that was similar to a falling leaf (Figure 4.3).

In the same sample set, cysts were also detected in feces on day two (Figure 4.5, Figure 4.6 and Figure 4.7). Figure 4.7 was taken under microscope with UV light and Figure 4.6 was taken without UV light. Suspicious *G. lamblia* cysts in Figure 4.7 were detected by conjugating them with anti-*Giardia* antibody and anti-mouse. In feces of sample set #3 on day 1 and day 2, suspicious cysts were detected (Figure 4.8). No evidence was found to prove that the particle shown in Figure 4.8 is a real *G. lamblia* cyst. It could either be a cyst or a non-cyst with similar shape and size.

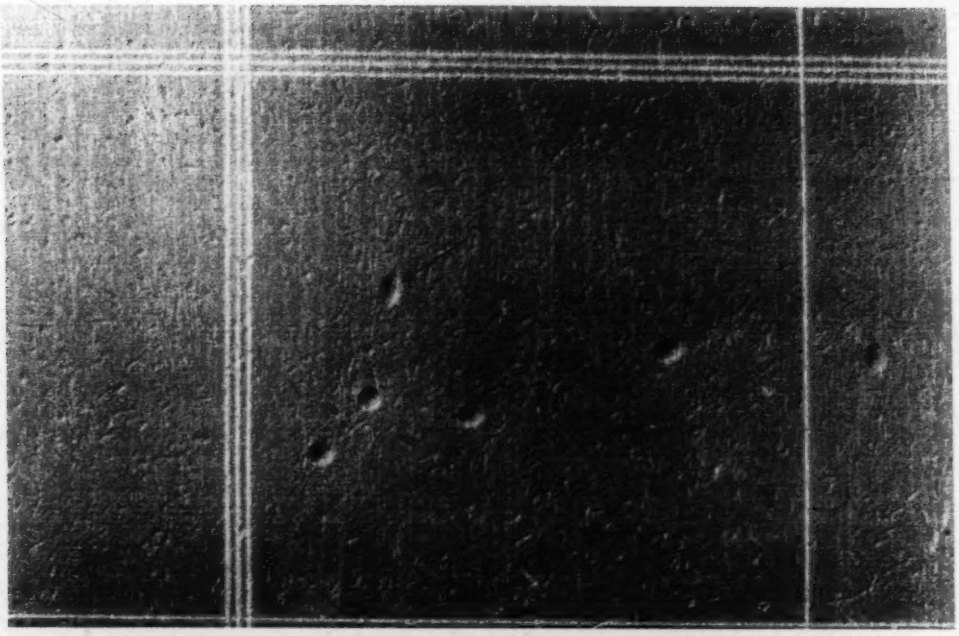


Figure 4. 5: *G. lamblia* cysts detected in feces check from gerbil number 8R on August 5 2003- City of Kelowna set #2.

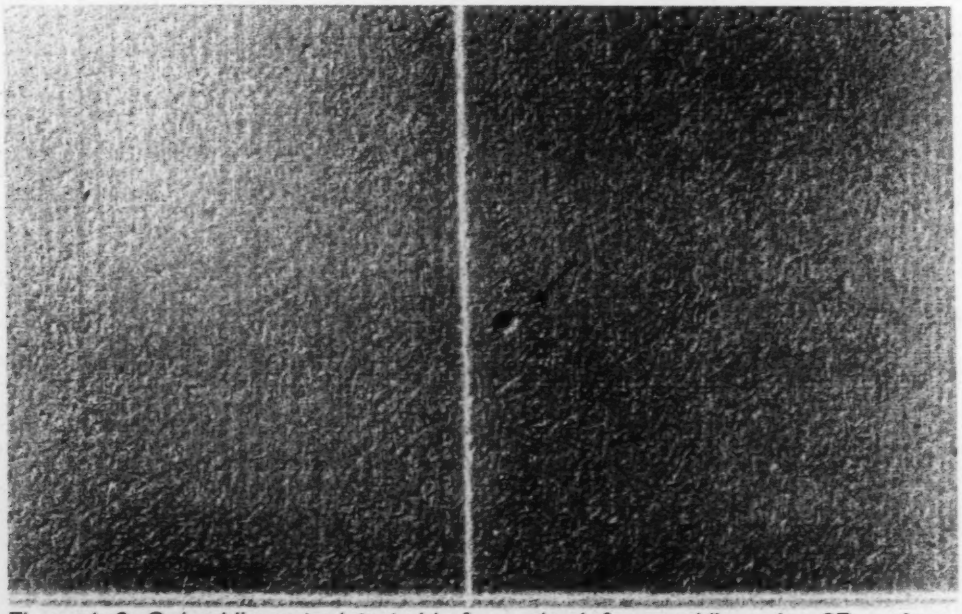


Figure 4. 6: *G. lamblia* cysts detected in feces check from gerbil number 2R on August 6 2003- City of Kelowna set #2.

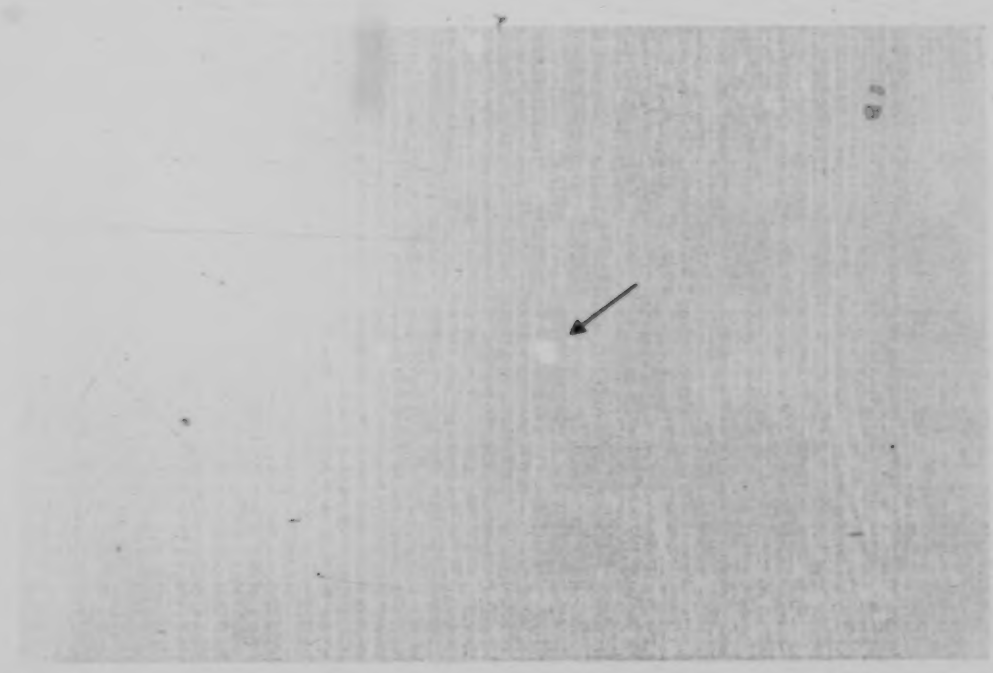


Figure 4. 7: *G. lamblia* cysts gathered from Gerbil #8R's feces check and conjugated with anti-*Giardia* antibody and anti-mouse,

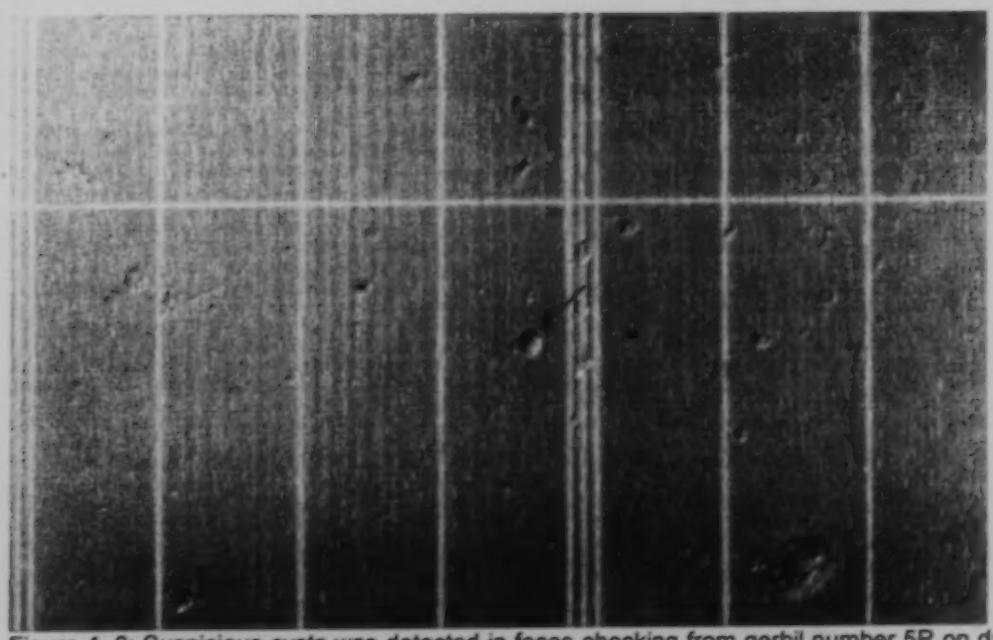


Figure 4. 8: Suspicious cysts was detected in feces checking from gerbil number 5R on day two-City of Kelowna set #3

Overall, gerbils inoculated with wastewater concentrates collected from upstream of the UV reactors seemed more likely to be infected and more likely to be heavily or moderately infected than those inoculated with wastewater concentrates collected from downstream of the UV reactors. Similar to the logistic regression analysis conducted for City of Edmonton, a logit model was fit to obtain the odds ratio of low or moderate infection for those gerbils inoculated with UV upstream samples to those inoculated with UV downstream samples.

Table 4.10 summarizes the statistical analysis results. The sample location effect was highly statistically significant (P-value = 0.003). The location where samples were collected did have an impact on the infection observed in the gerbils. The odds ratio of low or moderate infection was 6.72, with a confidence interval excluding 1. This odds ratio indicated that the gerbils inoculated with wastewater concentrates collected from upstream of the UV reactors were around 6.7 times more likely to be infected than those inoculated with concentrates collected from downstream.

Table 4. 10: Results from logistic regression analyses

Type III Analysis of Effects			
Effect	DF	Chi-Square	Pr > ChiSq
Sample location	1	8.8494	0.0029
Odds Ratio Estimates			
Effect	Point Estimate	95% Wald Confidence Limits	
Upstream vs Downstream	6.720	(1.915, 23.576)	

4.4.3 Summary of the Results

At both study WWTPs, *G. lamblia* cysts were detected in Mongolian gerbils inoculated with wastewater concentrates collected from upstream of the UV reactors. The breakdown of the total number of infected gerbils by the infectivity levels at these two study sites were similar. However, the severity of infection in individual gerbils scoring for positive for infection seemed slightly higher in City of Kelowna than in City of Edmonton. More than 56% of gerbils (14 out of 25) inoculated with samples collected from upstream of the UV reactors in City of Edmonton scored positive for moderate infection. In City of Kelowna, around 58% of gerbils (15 out of 26) inoculated with samples collected from upstream of the UV reactor were scored as moderately infected and one gerbil was scored as heavily infected.

Infections were also observed in gerbils inoculated with wastewater concentrates collected from downstream of the UV reactors installed at both study WWTPs. However, the intensity of infection scored was lower compared to the upstream samples at both study sites. Only 13% (3 out of 24) gerbils inoculated with UV downstream samples in City of Edmonton were detected as lowly infected. This figure was higher in City of Kelowna, which is 19% (5 out of 26).

As demonstrated in Table 4.11, there were slight differences between the distribution of infected gerbils in City of Edmonton and that in City of Kelowna. To test if the location of WWTP significantly affected the resulting infections, a logistic regression analysis was carried out in which both the location of WWTP and the sample location were included as explanatory variables. The location of WWTP was found to be insignificant (P-value = 0.48). Statistically, the infection rates of infection observed at the

two study sites were not different from each other. The sample location (i.e. upstream or downstream of the UV reactor) remained significant with a P-value 0.0001 (Table 4.12).

Table 4. 11: Comparison of infectivity results from City of Edmonton and City of Kelowna:

City	Sample	Total Number of Gerbils	Infectivity Level			
			-	+	++	+++
Edmonton	Downstream of UV Reactor	24	21	3	0	0
	Upstream of UV Reactor	26 (1 died before inoculation)	11	0	14	0
Kelowna	Downstream of UV Reactor	26	21	5	0	0
	Upstream of UV Reactor	26	10	0	15	1

1. "-", no infection; "+", low infection; "++", moderate infection, "+++", heavy infection.

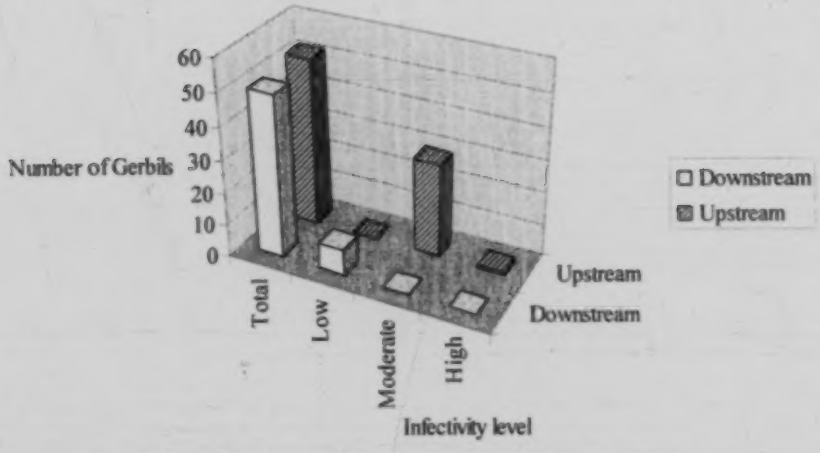
Table 4. 12: Significance of the location of WWTP

Type III Analysis of Effects			
Effect	DF	Chi-Square	Pr > ChiSq
Location of WWTP	1	0.4998	0.4796
Sample Location	1	17.7420	0.0001

Since the location of WWTP is an insignificant effect for interpreting resulting infections, data collected from the two study sites, City of Edmonton and City of Kelowna, were pooled and classified in terms of sample location only. The total number of gerbils inoculated with samples collected from upstream and downstream of the UV reactors, along with the number of gerbils scoring positive in each of the infection categories were combined and the results are summarized in Figure 4.9. The total number of infections in the upstream samples was greater than that of the downstream samples.

Thirty out of 52 gerbils inoculated with samples collected from upstream of the UV reactors scored positive for infection. Only 8 out 50 gerbils inoculated with samples collected from UV downstream scored positive for infection. A logistic regression analysis on the pooled data gave an odds ratio of 7.5. This implied that the odds of gerbils inoculated with upstream samples being infected was 7.5 times that of gerbils inoculated with downstream samples being infected. Moreover, the infections observed in upstream samples were also stronger than those observed in downstream samples. For the downstream samples, all of the gerbils scoring positive for infection were classified into the category of low infection. For the upstream samples, most of the infected gerbils scored positive for moderate infection and one gerbil scored positive for heavy infection.

The studies carried out in City of Edmonton and City of Kelowna proved that UV reactors operating under field conditions were effective for reducing the infectivity of *G. lamblia* cysts present naturally in wastewater effluents. The odds of gerbils that inoculated with downstream samples scored for infection was only 13% (1/7.5) of that of gerbils inoculated with upstream samples scoring for infection. However, the presence of lowly infected gerbils that inoculated with downstream samples suggested that UV treatment systems was not able to eliminate *G. lamblia* cysts infectivity.



	Total	Low	Moderate	High
Downstream	50	8	0	0
Upstream	52	0	29	1

Figure 4. 9: Comparison of number of infected gerbils inoculated with wastewater concentrates collected from upstream and downstream of UV reactors in the two cities of study

There are several possible explanations for the presence of low infections in gerbils inoculated with samples collected from downstream of the UV reactors at both study sites. Research carried out by Craik et al. (2000) suggested that the acute phase of infection of some parasites might be delayed when DNA of those parasites was damaged by UV radiation. Therefore, the multiplication rate of the trophozoite may be reduced and infected gerbils scored only for low infection. The ability of *Giardia* spp. to repair damaged DNA induced by UV radiation has been reported (Belosevic et al., 2001). Organisms can sometimes repair and reverse the destructive effects of UV through a

repair mechanism, known as photo reactivation, or in the absence of light known as dark repair. Because UV is a physical rather than a chemical disinfectant, it does not provide a residual to control pathogen proliferation and biofilm formation, therefore an inverse relationship exists between the applied UV dose and the ability of an organism to repair its damaged DNA. Once DNA is repaired, the ability of parasites to multiply will be restored, which may result in low infections observed in animals.

It is also possible that the low infections were the result of suspended solids, cyst clumps or aggregates or short circuiting in water flowing around UV reactors. Suspended solids can affect the disinfection efficiency of UV by shading bacteria and other pathogens, protecting them from UV radiation partially. Typically, the higher turbidities of wastewater can impact disinfection efficiency. For the same reason, clustering of *Giardia* cysts can also affect the disinfection efficiency of UV. Clusters of *G. lamblia* existing in wastewater concentrates before infectivity test had been observed (Figure 4.1 and 4.2). Clumped *G. l amblia* were also reported by Yakub et al. (2000). A study conducted by Medema et al. (1998b) showed that a significant proportion of (oo)cysts attempt to attach to biological particles in secondary effluent. The clustering of cysts can result in non-ideal exposure to UV in a reactor, and therefore, inadequate inactivation. Similar to particles that cause turbidity, microorganism aggregation can impact inactication efficiency by harboring pathogens within the aggregates and shade pathogens that would otherwise be inactivated. Poor geometry within the UV contactor (which creates spacing between lamps) can leave dead areas where inadequate disinfection occurs. As mentioned earlier, UV systems typically provide contact times on the order of

seconds. Therefore, short circuiting in the water flowing around UV reactors can result in inadequate exposure of parasites to UV and decrease the disinfection efficiency.

Another reason for the observed low infections may be due to the age of the cysts in the wastewater. Unlike the cysts used in laboratory for infectivity tests (Medema et al., 1998b), the cysts in wastewater may not be of the same age and may encounter different conditions. Therefore, a different level of DNA damage can be induced by UV radiation, and consequently, different infectivity levels maybe observed.

Other factors, such as the effects of fluence rate gradients in the reactors, experimental artefact related to identification of trophozoites in the intestine may also result in low infections.

Without additional data, it is difficult to sort out the real cause of low infections observed in the gerbils inoculated with wastewater concentrates collected from downstream of the UV reactors. It is even more difficult to interpret the public health significance of these low infections. UV treatment may not be able to eliminate the infectious potential of *G. lambia* cysts present in the treated wastewater. Nevertheless, the studies conducted in City of Edmonton and City of Kelowna provided evidence of the effectiveness of UV treatment for reducing the infectious potential of *G. lambia* cysts present in the treated wastewater.

Laboratory research work revealed that a 2 to 3 log inactivation of *Giardia* cysts could be achieved with a UV dose 5 to 83 mJ/cm^2 (Craik et al., 2000). The calculated UV doses at the sample sites in City of Edmonton and City of Kelowna are 30 mJ/cm^2 and 50 mJ/cm^2 respectively. Therefore, the expected inactivation of *G. lamblia* at the two WWTPs was most likely 2 to 3 log as well.

The recovery experiments described in Chapter 3 (section 3.4) revealed that the wastewater matrix contributes to a much lower recovery than deionized water does. The recovery rate of cysts in the wastewater samples was 5.85%, which is much lower than that observed in deionized water, 17.3%. Therefore, the real number of infectious cysts in wastewater samples should be greater than what had been observed (Table 4.11).

Because of the clustering of cysts (Figure 4.2), it was not possible to enumerate the cysts in the wastewater concentrates. If the clustered cysts can be enumerated, the concentration of infectious cysts in wastewater samples would be known. It is then possible to find out the significance of the clustering phenomena on the efficiency of inactivation in the field UV reactors by comparing the resulting infections of gerbils inoculated with wastewater samples of different concentrations or difference size of clusters of cysts.

5 CONCLUSION

The presence of significant concentration *G. lamblia* cysts and *C. parvum* oocysts in effluents discharged from municipal wastewater treatment plants has been shown in many studies carried out in the past decade. Some studies reported that infectious cysts and oocysts had been detected in wastewater effluents. Wastewater treatments are designed to inactivate or eliminate these infectious (oo)cysts and other disease-causing micro-organisms from water before discharge into receiving water body. Investigations on effective wastewater disinfection systems for wastewater effluents have become significant to public health.

UV irradiation has become a commonly used wastewater disinfection technique in North America. In controlled laboratory experiments, UV has demonstrated to be effective against *G. lamblia* cysts and *C. parvum* when purified preparations of parasites suspended in clean water are exposed to relatively low doses. However, the effectiveness of UV treatment reactors operating under field conditions against *G. lamblia* cysts and *C. parvum* present naturally in wastewater effluents has rarely been studied.

In this study, the concentration and the infectivity of *G. lamblia* present in UV treated municipal wastewater concentrates from two wastewater treatment plants in Western Canada were examined. The Miltenyi Biotach IMS system with flow cytometry/FACTS has been successfully used to concentrate and detect infectious *G. lamblia* cysts present in drinking water. However, Miltenyi Biotach IMS system is not suitable for purification of turbid and large volume of wastewater concentration since the separation columns become plugged with suspended solids in wastewater at this condition. The flow cytometry/FACS can fail to detect the oocysts because the direct

antibody used may result in clustering of oocysts at the higher concentrations which are likely to be encountered in wastewater. A modified procedure adapted from Miltenyi Biotach IMS system with flow cytometry/FACS was developed to concentrate and detecte *G. lamblia* in wastewater effluents. In this modified procedure, the IMS was replaced by sucrose flotation and the flow cytometry/FACTS was substituted by microscope detection. Mongolian gerbil model was then employed to determine the infectivity of *G. lamblia.*

The suitability of the Miltenyi Biotach IMS system with flow cytometry/FACTS for recovering and enumerating cysts and oocysts present in treated municipal wastewater was evaluated by spike tests. The recovery of prepared *C. parvum* oocysts spiked into deionised water was measured using the Miltenyi Biotach IMS system with flow cytometry/FACTS. The recovery rate of oocysts was very low when the level of concentration of oocysts is close to that in treated wastewater. Clumps of oocysts were observed and they were not able to be detected by flow cytometry because their sizes exceeded the defined gate region of flow cytometry. Studies carried out showed that the key factor for the forming of clumps was the direct antibody. The recovery of prepared and immunofluorescently-labelled *G. lamblia* cysts was measured using a modified method in which the Miltenyi Biotech IMS purification procedure was replaced with sucrose flotation. The recovery rate of cysts in wastewater samples collected from Edmonton Goldbar WWTP was 5.85%, which was much lower than that observed in deionized water, 17.3%, but sufficient to carry out infectivity analysis.

In the infectivity analysis, wastewater concentrates were collected upstream and downstream of the UV reactors at Edmonton Goldbar WWTP and the WWTP in City of

Kelowna. For City of Edmonton, only 3 out 24 gerbils inoculated with wastewater concentrates collected from downstream of the UV reactors were detected as lowly infectious. The rest scored for no infection. In the case of gerbils being inoculated with wastewater concentrates collected from upstream of the UV reactors, over half (14 out 26) gerbils scored positive for moderate infection, eleven scored no infection and one died before inoculation. Similar results were observed in City of Kelowna. Only 5 out 26 gerbils inoculated with UV downstream samples were detected as lowly infectious. The rest scored for no infection. Among the 26 gerbils inoculated with UV upstream samples, fifteen scored for moderate infection and one for heavy infection. No infection was observed for the rest 10 gerbils.

Infection in Mongolian gerbils suggested that live and infectious *G. lamblia* cysts were present in the wastewater both upstream and downstream of the UV reactors at both study WWTPs. Statistical analysis provided no evidence for a significant effect of WWTP location. As a result, data collected from the two sites were pooled for analyses.

UV treatment was effective in reducing the infectious potential of *G. lamblia* cysts present in the treated wastewater. Out 52 gerbils inoculated with wastewater concentrates recovered from upstream of UV reactors at WWTPs of study, 29 (56%) were moderately infected and 1 was heavily infected. The number of infected gerbils declined to 8 (out 50) when gerbils were inoculated with wastewater concentrates collected from downstream of the UV reactors. Further more, the severity of infection also decrease in the UV downstream samples. All the 8 infections observed were classified into the category of low infection. Statistical analysis provided strongly evidence for significant effect of the sample location. The odds of gerbils that inoculated

with UV downstream samples scored positive for infection was only 13% to that of gerbils inoculated with UV upstream samples. The low but non zero odds ratio implied that UV treatment operating in field conditions was effective in reducing, but not eliminating, the infectious potential of *G. lamblia* cysts in the treated wastewater.

Many factors can cause the low infection observed in UV downstream samples. Physically, suspended solids, forming of clump or aggregation in water flowing through the UV contactor can block cysts from UV irradiation and result in incomplete inactivation. Biologically, the DNA repair of the cysts can decrease the inactivation efficiency and the age of cysts can result in different levels of infection. Other factors, such as the effects of fluence rate gradients in the reactors, or experimental artefact may also result in low infections.

The presence of infectious cysts has been proved by the infectivity test using the Mongolian gerbil model. However, no cysts were detected in any of the wastewater concentrated by flow cytometry. Microscopic examination demonstrated that cysts present in purified wastewater concentrates tended to form clusters. The size of these clusters of cysts (possible infectious) was too large to be captured in the defined gate region of flow cytometry. As a result, cysts in wastewater concentrates could not be enumerated. Without further data, it is difficult to conclude the significance of the clustering phenomena on the efficiency of UV reactors against *G. lamblia* cysts.

The recovery rate of cysts in wastewater was determined as 5.85% using spiked tests. It was much lower than that of cysts in deionized water, which is believed to result from the particular wastewater matrix. It suggested that the real number of infectious

cysts in wastewater samples was likely greater than that resulted from the infectivity analysis using Mongolian gerbil model.

RECOMMENDATIONS

In this study, the Miltenyi Biotech IMS separation column was often plugged by the particles present in the wastewater samples and failed to separate *C. parvum* and *G. lamblia* from the wastewater concentrates. In further studies, it is recommended that the Dynal IMS separation system, developed by DynCorp, be substituted for the Miltenyi Biotech IMS separation system. The Dynal technique employs a separation tube to recover protozoan parasites from samples rather than a separation column that is easily plugged with particles. The efficiency of the separation of *Cryptosporidium* oocysts from wastewater samples using the Dynal IMS system was reported to be 32.6% for raw sewage, 38.8% for primary effluent, 53% for secondary effluent, and 67.8% for tertiary effluent (McCuin et al.,2002). These recoveries are greater than the 5.85% recovery attained by using the 1M sucrose purification process and reported in this thesis. The Dynal IMS system may possibly yield improved *Giardia* cysts recovery relative to the sucrose method.

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7 APPENDIX

7.1 Raw Data

7.1.1 Raw Data for City Edmonton

Table 7. 1: *Giardia lamblia* cysts detection by samples from GB on Month: 09 Day: 08Year: 2003Filtration Date: Aug.26/2003Infection Date: Aug.29/2003

Cage #	Sample source	Tube #	Weight 1	Weight 2	∇ Weight	Count 1	Count 2	Count 3	Count 4
1	downstream	1R	6.43	7.58	1.15	-	-	-	-
		1N	6.44	7.54	1.10	-	-	-	-
2	upstream	2R	6.40	7.15	0.75	-	-	-	-
		2N	6.82	7.73	0.91	+	+	+	+
3	downstream	3R	6.83	7.98	1.15	-	-	-	-
		3N	6.83	8.15	1.32	-	-	-	-
4	upstream	4R	6.70	8.00	1.30	-	-	-	-
		4N	6.68	8.15	1.47	-	-	-	-
5	downstream	5R	6.72	7.76	1.04	-	-	-	-
		5N	6.70	8.25	1.55	-	-	-	-
6	upstream	6R	6.71	8.28	1.57	-	-	-	-
		6N	6.61	7.85	1.24	-	-	-	-
7	downstream	7R	6.70	7.23	0.53	-	-	-	-
		7N	6.20	7.89	1.69	-	-	-	-
8	upstream	8R	6.79	7.86	1.07	-	-	-	-
		8N	6.76	8.01	1.25	-	-	-	-
9	upstream	9R	6.73	7.85	1.12	-	-	-	-
		9N	6.64	7.85	1.21	-	-	-	-
10	Negative control	10R	6.60	7.80	1.20	-	-	-	-
		10N	6.60	7.74	1.14	-	-	-	-
11	Negative control	11R	6.75	8.52	1.77	-	-	-	-
		11N	6.66	8.05	1.39	-	-	-	-

Note: Gerbils were placed on false bottom at 9:30am and feces were collected in 1 hour (10:30)

Table 7. 2: *Giardia lamblia* cysts detection by samples from GB on Month: 09 Day: 09 Year: 2003
Filtration Date: Aug.26/2003 Infection Date: Aug.29/2003

Cage #	Sample source	Tube #	Weight 1	Weight 2	∇ Weight	Count 1	Count 2	Count 3	Count 4
1	downstream	1R	6.99	7.76	0.77	-	-	-	-
		1N	6.98	7.86	0.88	-	-	-	-
2	upstream	2R	7.50	9.10	1.60	-	-	-	-
		2N	7.56	8.04	0.48	-	-	-	-
3	downstream	3R	7.66	8.81	1.15	-	-	-	-
		3N	7.07	8.31	1.24	-	-	-	-
4	upstream	4R	6.73	7.82	1.09	-	-	-	-
		4N	6.65	7.90	1.25	-	-	-	-
5	downstream	5R	7.15	8.27	1.12	-	-	-	-
		5N	7.39	8.37	0.98	-	-	-	-
6	upstream	6R	6.70	8.39	1.69	-	-	-	-
		6N	7.02	8.17	1.15	+	-	+	-
7	downstream	7R	6.64	7.56	0.92	-	-	-	-
		7N	6.85	7.85	1.00	-	-	-	-
8	upstream	8R	7.34	8.60	1.26	-	-	-	-
		8N	7.70	7.83	0.13	-	-	-	-
9	upstream	9R	7.20	8.58	1.38	-	-	-	-
		9N	7.02	7.26	0.24	-	-	-	-
10	Negative control	10R	6.66	7.85	1.19	-	-	-	-
		10N	6.76	7.75	0.99	-	-	-	-
11	Negative control	11R	6.81	8.10	1.29	-	-	-	-
		11N	6.86	8.04	1.18	-	-	-	-

Note: Gerbils were placed on false bottom at 9:30am and feces were collected in 1 hour (10:30)

Table 7. 3: *Giardia lamblia* cysts detection by samples from GB on Month: 09 Day: 10Year: 2003
Filtration Date: Aug.26/2003 Infection Date: Aug.29/2003

Cage #	Sample source	Tube #	Weight 1	Weight 2	∇ Weight	Count 1	Count 2	Count 3	Count 4
1	downstream	1R	6.70	N/A*	N/A*	-	-	-	-
		1N	6.63	7.67	1.04	-	-	-	-
2	upstream	2R	6.76	7.75	0.99	-	-	-	-
		2N	6.59	7.52	0.93	-	-	-	-
3	downstream	3R	6.58	7.38	0.80	-	-	-	-
		3N	6.68	7.45	0.77	-	-	-	-
4	upstream	4R	6.63	7.60	0.97	-	-	-	-
		4N	6.85	8.20	1.35	-	-	-	-
5	downstream	5R	6.63	7.08	0.45	-	-	-	-
		5N	6.77	7.75	0.98	-	-	-	-
6	upstream	6R	6.59	7.61	1.02	-	-	-	-
		6N	6.64	7.52	0.88	-	-	-	-
7	downstream	7R	6.70	7.64	0.94	-	-	-	-
		7N	6.59	7.53	0.94	-	-	-	-
8	upstream	8R	6.58	7.53	0.95	-	-	-	-
		8N	6.74	7.88	1.14	-	-	-	-
9	upstream	9R	6.62	7.62	1.00	-	-	-	-
		9N	6.59	7.37	0.78	-	-	-	-
10	Negative control	10R	6.62	7.75	1.13	-	-	-	-
		10N	6.61	7.73	1.12	-	-	-	-
11	Negative control	11R	6.65	8.26	1.61	-	-	-	-
		11N	6.83	7.77	0.94	-	-	-	-

Note: Gerbils were placed on false bottom at 9:30am and feces were collected in 1 hour (10:30)

N/A*: Weight for sample 1R is not available, milliQ water was added before the sample was weighted.

Table 7. 4: Trophozoite detection by samples from GB on Month: 09 Day: 11Year: 2003 Filtration Date: Aug.26/2003 Infection Date: Aug.29/2003

Cage#	Sample Source	Tube#	Tube# Continue					
			A		B		C	D
			Count1	Count2	Count1	Count2		
1	downstream	1R	-	-	-	-	-	-
		1N	-	-	-	-	-	-
2	upstream	2R	++	-	-	-	-	-
		2N	++	-	++	-	-	-
3	downstream	3R	-	-	-	-	-	-
		3N	-	-	-	-	-	-
4	upstream	4R	-	-	-	-	-	-
		4N	-	-	-	++	-	-
5	downstream	5R	-	-	-	-	-	-
		5N	-	-	-	-	-	-
6	upstream	6R	-	-	-	-	-	-
		6N	-	-	-	-	-	-
7	downstream	7R	-	-	-	-	-	-
		7N	-	-	-	-	-	-
8	upstream	8R	-	-	-	-	-	-
		8N	-	-	-	-	-	-
9	upstream	9R	-	-	-	-	-	-
		9N	-	-	-	-	-	-
10	Negative control	10R	-	-	-	-	-	-
		10N	-	-	-	-	-	-
11	Negative control	11R	-	-	-	-	-	-
		11N	-	-	-	-	-	-

Table 7. 5: *Giardia lamblia* cysts detection by samples from GB on Month: 08 Day: 25Year: 2003
Filtration Date: Aug.12/2003 Infection Date: Aug.15/2003

Cage #	Sample source	Tube #	Weight 1	Weight 2	∇ Weight	Count 1	Count 2	Count 3	Count 4
1	downstream	1R	6.54	7.20	0.66	-	-	-	-
		1N	6.71	7.18	0.47	-	-	-	-
2	upstream	2R	6.65	7.41	0.76	-	-	-	-
		2N	6.77	7.18	0.41	-	-	-	-
3	downstream	3R	7.14	7.89	0.75	-	-	-	-
		3N	7.01	7.67	0.66	-	-	-	-
4	upstream	4R	7.19	7.91	0.72	-	-	-	-
		4N	7.20	8.01	0.81	-	-	-	-
5	downstream	5R	7.23	7.96	0.73	-	-	-	-
		5N	6.81	7.33	0.52	-	-	-	-
6	upstream	6R	7.25	7.84	0.59	-	-	-	-
		6N	7.18	7.80	0.62	-	-	-	-
7	Positive control	7R	6.53	7.45	0.92	-	-	-	-
		7N	6.75	7.22	0.47	-	-	-	-
8	Negative control	8R	7.05	7.79	0.74	-	-	-	-
		8N	6.82	7.44	0.62	-	-	-	-

Table 7. 6: *Giardia lamblia* cysts detection by samples from GB on Month: 08 Day: 26Year: 2003
Filtration Date: Aug.12/2003 Infection Date: Aug.15/2003

Cage #	Sample source	Tube #	Weight 1	Weight 2	∇ Weight	Count 1	Count 2	Count 3	Count 4
1	downstream	1R	6.82	8.17	1.35	-	-	-	-
		1N	7.12	9.00	1.88	-	-	-	-
2	upstream	2R	7.08	8.43	1.35	-	-	-	-
		2N	6.78	7.96	1.18	-	-	-	-
3	downstream	3R	6.94	8.70	1.76	-	-	-	-
		3N	7.13	8.51	1.38	-	+	-	-
4	upstream	4R	7.36	8.04	0.68	-	-	-	-
		4N	6.80	7.48	0.68	-	-	-	-
5	downstream	5R	6.44	7.86	1.42	-	-	-	-
		5N	7.24	8.26	1.02	-	-	-	-
6	upstream	6R	6.92	7.75	0.83	-	-	-	-
		6N	6.71	7.75	1.04	-	-	-	-
7	Positive control	7R	7.17	7.40	0.23	-	-	-	-
		7N	7.08	7.86	0.78	-	-	-	-
8	Negative control	8R	6.56	7.22	0.66	-	-	-	-
		8N	7.08	7.45	0.37	-	-	-	-

Table 7. 7: *Giardia lamblia* cysts detection by samples from GB on Month: 08 Day: 27Year: 2003
Filtration Date: Aug.12/2003 Infection Date: Aug.15/2003

Cage #	Sample source	Tube #	Weight 1	Weight 2	∇ Weight	Count 1	Count 2	Count 3	Count 4
1	downstream	1R	7.61	8.73	1.12	-	-	-	-
		1N	6.68	7.72	1.04	-	-	-	-
2	upstream	2R	7.23	8.82	1.59	-	+*	-	-
		2N	7.03	8.42	1.39	-	-	-	-
3	downstream	3R	6.99	8.19	1.20	-	-	-	-
		3N	6.53	7.85	1.32	-	-	-	-
4	upstream	4R	6.97	7.69	0.72	-	-	-	-
		4N	7.26	8.26	1.00	-	-	-	-
5	downstream	5R	7.28	8.33	1.05	-	-	-	-
		5N	7.15	8.04	0.89	-	-	-	-
6	upstream	6R	6.80	7.45	0.65	-	-	-	-
		6N	7.13	8.14	1.01	-	-	-	-
7	Positive control	7R	6.94	7.39	0.45	-	-	-	-
		7N	6.74	8.04	1.30	-	-	-	-
8	Negative control	8R	7.24	8.05	0.81	-	-	-	-
		8N	7.22	8.14	0.92	-	-	-	-

+*: Similar shape as *Giardia lamblia*, but smaller in size and had a glossy outer layer.

Table 7. 8: Trophozoite detection by samples from GB on Month: 08 Day: 28Year: 2003 Filtration Date: Aug.12/2003 Infection Date: Aug.15/2003

Cage#	Sample Source	Tube#	Tube# Continue					
			A		B		C	D
			Count1	Count2	Count1	Count2		
1	downstream	1R	-	-	-	-	-	-
		1N	-	-	-	-	-	-
2	upstream	2R	-	-	-	-	-	-
		2N	-	-	-	-	-	-
3	downstream	3R	-	-	-	-	-	-
		3N	-	-	+	+	-	-
4	upstream	4R	++	++	-	-	-	-
		4N	-	-	++	++	-	-
5	downstream	5R	+	+	-	-	-	-
		5N	+	+	+	+	-	-
6	upstream	6R	-	-	++	-	-	-
		6N	-	-	++	-	-	-
7	Positive control	7R	-	-	-	-	-	-
		7N	++	++	++	++	-	-
8	Negative control	8R	-	-	-	-	-	-
		8N	-	-	-	-	-	-

Table 7. 9: *Giardia lamblia* cysts detection by samples from GB on Month: 06 Day: 23Year: 2003
Filtration Date: June 10/2003 Infection Date: June 13/2003

Cage #	Sample source	Tube #	Weight 1	Weight 2	∇ Weight	Count 1	Count 2	Count 3	Count 4
1	downstream	1R	6.43	7.36	0.93	-	+*	-	-
		1N	7.49	8.17	0.68	-	-	-	-
2	upstream	2R	6.63	7.66	1.03	-	-	-	-
		2N	6.66	7.23	0.57	-	-	-	-
3	downstream	3R	6.55	7.42	0.87	-	-	-	-
		3N	6.69	7.41	0.72	-	-	-	-
4	upstream	4R	6.72	7.36	0.64	-	-	-	-
		4N							
5	downstream	5R	6.70	7.84	1.14	-	-	-	-
		5N	6.63	7.06	0.43	-	-	-	-
6	upstream	6R	6.69	7.49	0.80	-	-	-	-
		6N	6.66	7.17	0.51	-	-	-	-
7	downstream	7R	6.67	7.49	0.82	-	-	-	-
		7N	6.65	7.57	0.92	-	-	-	-
8	upstream	8R	6.74	7.59	0.85	-	-	-	-
		8N	6.79	7.82	1.03	-	-	-	-
9	downstream	9R	6.67	7.25	0.58	-	-	-	-
		9N	6.66	7.28	0.62	-	-	-	-
10	upstream	10R	6.74	7.42	0.68	-	-	-	-
		10N	6.70	7.73	1.03	-	-	-	-

Note: Gerbils were placed on false bottom at 9:30am and feces were collected in 1 hour (10:30)
+*: Similar shape as *Giardia lamblia*, but smaller in size and had a glossy outer layer. Mostly like glove contamination.
Gerbil #4N was dead during the experiment.

Table 7. 10: *Giardia lamblia* cysts detection by samples from GB on Month: 06 Day: 24Year: 2003
Filtration Date: June 10/2003 Infection Date: June 13/2003

Cage #	Sample source	Tube #	Weight 1	Weight 2	∇ Weight	Count 1	Count 2	Count 3	Count 4
1	downstream	1R	6.60	7.51	0.91	-	-	-	-
		1N	6.44	7.33	0.89	-	-	-	-
2	upstream	2R	6.67	7.49	0.82	-	-	-	-
		2N	6.48	7.10	0.62	-	-	-	-
3	downstream	3R	6.63	7.47	0.84	-	-	-	-
		3N	6.64	7.16	0.52	-	-	-	-
4	upstream	4R	6.70	7.08	0.38	-	-	-	-
		4N							
5	downstream	5R	6.70	7.54	0.84	-	-	-	-
		5N	6.68	7.55	0.87	-	-	-	-
6	upstream	6R	6.69	7.64	0.95	-	-	-	-
		6N	6.66	7.53	0.87	-	-	-	-
7	downstream	7R	6.67	7.78	1.11	-	-	-	-
		7N	6.65	7.53	0.88	-	-	-	-
8	upstream	8R	6.72	7.31	0.59	-	-	-	-
		8N	6.69	7.65	0.96	-	-	-	-
9	downstream	9R	6.67	7.28	0.61	-	-	-	-
		9N	6.65	7.58	0.93	-	-	-	-
10	upstream	10R	6.72	7.07	0.35	-	-	-	-
		10N	6.70	7.52	0.82	-	-	-	-

Note: Gerbils were placed on false bottom at 9:30am and feces were collected in 1 hour (10:30)
Gerbil #4N was dead during the experiment.

Table 7. 11: *Giardia lamblia* cysts detection by samples from GB on Month: 06 Day: 25Year: 2003
Filtration Date: June 10/2003 Infection Date: June 13/2003

Cage #	Sample source	Tube #	Weight 1	Weight 2	∇ Weight	Count 1	Count 2	Count 3	Count 4
1	downstream	1R	6.44	7.32	0.88	-	-	-	-
		1N	6.36	7.48	1.12	-	-	-	-
2	upstream	2R	6.52	7.66	1.14	-	-	-	-
		2N	6.72	7.76	1.04	-	-	-	-
3	downstream	3R	6.70	7.49	0.79	-	-	-	-
		3N	6.66	7.23	0.57	-	-	-	-
4	upstream	4R	6.45	7.36	0.91	-	-	-	-
		4N							
5	downstream	5R	6.46	7.28	0.82	-	-	-	-
		5N	6.50	7.89	1.39	-	-	-	-
6	upstream	6R	6.56	7.69	1.13	-	-	-	-
		6N	6.66	7.31	0.65	-	-	-	-
7	downstream	7R	6.72	7.51	0.79	-	-	-	-
		7N	6.67	7.33	0.66	-	-	-	-
8	upstream	8R	6.79	7.65	0.86	-	-	-	-
		8N	6.50	7.44	0.94	-	-	-	-
9	downstream	9R	6.66	7.20	0.54	-	-	-	-
		9N	6.46	7.21	0.75	-	-	-	-
10	upstream	10R	6.44	7.40	0.96	-	-	-	-
		10N	6.65	7.17	0.52	-	-	-	-

Note: Gerbils were placed on false bottom at 8:30am and feces were collected in 1 hour (9:30)

Table 7. 12: Trophozoite detection by samples from GB on Month: 06 Day: 26/27Year: 2003
Filtration Date: June 10/2003 Infection Date: June 13/2003

Cage#	Sample Source	Tube#	Tube# Continue					
			A		B		C	D
			Count1	Count2	Count1	Count2		
1	downstream	1R	-	-	-	-	-	-
		1N	-	-	-	-	-	-
2	upstream	2R	++	++	++	++	-	-
		2N	++	++	++	++	-	-
3	downstream	3R	-	-	-	-	-	-
		3N	-	-	-	-	-	-
4	upstream	4R	++	++	++	++	-	-
		4N						
5	downstream	5R	-	-	-	-	-	-
		5N	-	-	-	-	-	-
6	upstream	6R	++	++	++	++	-	-
		6N	++	++	++	++	-	-
7	downstream	7R	-	-	-	-	-	-
		7N	-	-	-	-	-	-
8	upstream	8R	++	++	++	++	-	-
		8N	++	++	++	++	-	-
9	downstream	9R	-	-	-	-	-	-
		9N	-	-	-	-	-	-
10	upstream	10R	-	-	-	-	-	-
		10N	-	-	-	-	-	-

Note: Downstream was checked on June 26/2003, upstream was checked on June 27/2003.

Gerbile#4N was dead during the experiment.

7.1.2 Raw Data for City Kelowna

Table 7. 13: *Giardia lamblia* cysts detection by samples from KL on Month: 09 Day: 02Year: 2003
Filtration Date: Aug.18/19/2003 Infection Date: Aug.22/2003

Cage #	Sample source	Tube #	Weight 1	Weight 2	∇ Weight	Count 1	Count 2	Count 3	Count 4
1	downstream	1R	6.36	6.68	0.32	-	-	-	-
		1N	6.42	7.47	1.05	-	-	-	-
2	upstream	2R	6.37	7.49	1.12	-	-	-	-
		2N	6.41	7.02	0.61	-	-	-	-
3	downstream	3R	6.43	8.00	1.57	-	-	-	-
		3N	6.46	7.34	0.88	-	-	-	-
4	upstream	4R	6.39	7.52	1.13	-	-	-	-
		4N	6.42	7.34	0.92	-	-	-	-
5	downstream	5R	6.37	8.00	1.63	-	-	-	-
		5N	6.40	8.10	1.70	+?	-	-	-
6	upstream	6R	6.44	8.06	1.62	-	-	-	-
		6N	6.41	7.83	1.42	-	-	-	-
7	Negative	7R	6.44	7.89	1.45	-	-	-	-
		7N	6.39	8.02	1.63	-	-	-	-

Note: Gerbils were placed on false bottom at 9:30am. And feces were collected one hour later.
+?: looks like cysts and was photographed.

Table 7. 14: *Giardia lamblia* cysts detection by samples from KL on Month: 09 Day: 03Year: 2003
Filtration Date: Aug.18/19/2003 Infection Date: Aug.22/2003

Cage #	Sample source	Tube #	Weight 1	Weight 2	∇ Weight	Count 1	Count 2	Count 3	Count 4
1	downstream	1R	7.02	7.86	0.84	-	-	-	-
		1N	6.99	7.64	0.65	-	-	-	-
2	upstream	2R	7.02	8.12	1.10	+?	-	+?	+?
		2N	6.98	7.85	0.87	-	-	-	-
3	downstream	3R	7.00	8.44	1.44	-	-	-	-
		3N	7.05	7.85	0.80	-	-	-	-
4	upstream	4R	7.02	7.80	0.78	-	-	-	-
		4N	7.01	8.09	1.08	-	-	-	-
5	downstream	5R	7.02	7.76	0.74	-	+?	-	-
		5N	7.08	8.32	1.24	-	-	-	-
6	upstream	6R	6.98	8.74	1.76	-	-	-	-
		6N	7.06	8.08	1.02	-	-	-	-
7	Negative	7R	6.99	8.51	1.52	-	-	-	-
		7N	6.98	8.03	1.05	-	-	-	-

Note: Gerbils were placed on false bottom at 9:30am. And feces were collected one hour later.
+?: looks like cysts and was photographed.

Table 7. 15: *Giardia lamblia* cystsdetection by samples from KL on Month: 09 Day: 04Year: 2003
Filtration Date: Aug.18/19/2003 Infection Date: Aug.22/2003

Cage #	Sample source	Tube #	Weight 1	Weight 2	∇ Weight	Count 1	Count 2	Count 3	Count 4
1	downstream	1R	6.60	7.08	0.48	-	-	-	-
		1N	6.55	7.42	0.87	-	-	-	-
2	upstream	2R	7.06	7.94	0.88	-	-	-	-
		2N	6.63	7.08	0.45	-	-	-	-
3	downstream	3R	6.66	7.82	1.16	-	-	-	-
		3N	6.45	7.34	0.89	-	-	-	-
4	upstream	4R	6.66	7.42	0.76	-	-	-	-
		4N	6.63	7.63	1.00	-	-	-	-
5	downstream	5R	6.59	7.55	0.96	-	-	-	-
		5N	6.58	7.86	1.28	-	-	-	-
6	upstream	6R	6.64	7.91	1.27	-	-	-	-
		6N	6.55	7.52	0.97	-	-	-	-
7	Negative	7R	6.58	6.90	0.32	-	-	-	-
		7N	6.67	7.89	1.22	-	-	-	-

Note: Gerbils were placed on false bottom at 9:30am. And feces were collected one hour later.

Table 7. 16: Trophozoite detection by samples from KL on Month: 09 Day: 04Year: 2003 Filtration Date: Aug. 18,19/2003 Infection Date: Aug.22/2003

Cage#	Sample Source	Tube#	Tube# Continue					
			A		B		C	D
			Count1	Count2	Count1	Count2		
1	downstream	1R	-	-	-	-	-	-
		1N	-	-	-	-	-	-
2	upstream	2R	-	-	-	-	-	-
		2N	-	-	-	-	-	-
3	downstream	3R	-	-	-	-	-	-
		3N	-	-	-	-	-	-
4	upstream	4R	-	-	-	-	-	-
		4N	-	-	-	-	-	-
5	downstream	5R	+*	-	-	-	-	-
		5N	+*	-	+*	-	-	-
6	upstream	6R	++	-	-	-	-	-
		6N	-	-	-	-	-	-
7	Negative control	7R	-	-	-	-	-	-
		7N	-	-	-	-	-	-

+*: Suspected Trophozoites.

Table 7. 17: *Giardia lamblia* cysts detection by samples from KL on Month: 08 Day: 05Year: 2003
Filtration Date: July 21,22/2003 Infection Date: July 25/2003

Cage #	Sample source	Tube #	Weight 1	Weight 2	∇ Weight	Count 1	Count 2	Count 3	Count 4
1	downstream	1R	6.64	6.90	0.26	-	-	-	-
		1N	6.61	7.11	0.50	-	-	-	-
2	upstream	2R	6.59	7.09	0.50	-	-	-	-
		2N	6.57	6.93	0.36	-	-	-	-
3	downstream	3R	6.61	6.94	0.33	-	-	-	-
		3N	6.61	7.01	0.40	-	-	-	-
4	upstream	4R	6.39	6.89	0.50	-	-	-	-
		4N	6.59	7.00	0.41	-	-	-	-
5	downstream	5R	6.60	7.42	0.82	-	-	-	-
		5N	6.61	7.17	0.56	-	-	-	-
6	upstream	6R	6.54	6.95	0.41	-	-	-	-
		6N	6.59	7.83	1.24	+*	-	-	-
7	downstream	7R	6.55	6.65	0.10	-	-	-	-
		7N	6.61	7.28	0.67	-	-	-	-
8	upstream	8R	6.49	6.88	0.39	+++66	+++63	+++82	+++72
		8N	6.64	7.51	0.87	-	-	-	-
9	downstream	9R	6.57	7.48	0.91	-	-	-	-
		9N	6.63	7.42	0.79	-	-	-	-
10	upstream	10R	6.60	7.60	1.00	-	-	-	-
		10N	6.61	7.68	1.07	-	-	-	-

Note: Gerbils were placed on false bottom at 9:45am.
+* sample 6N suspect-smaller than regular *Giardia lamblia* cyst. Outer membrane rigid.
+++66,+++63,+++82,+++72 *Giardia Lamblia* cysts present. Counts 66,63,82,72
#=(66+63+82+72)/4*10000/4=176,875/ml
+: Parasites observed were dead, no flagella movement, observed by Dr. Belosevic (downstream only).
++: Parasites observed with flagella movement.
+++: Heavily infected.

Table 7. 18: *Giardia lamblia* cysts detection by samples from KL on Month: 08 Day: 06Year: 2003
Filtration Date: July 21,22/2003 Infection Date: July 25/2003

Cage #	Sample source	Tube #	Weight 1	Weight 2	∇ Weight	Count 1	Count 2	Count 3	Count 4
1	downstream	1R	6.47	7.04	0.57	-	-	-	-
		1N	7.02	7.78	0.76	-	-	-	-
2	upstream	2R	6.54	7.44	0.9	-	-	-	-
		2N	6.53	7.63	1.10	-	-	-	-
3	downstream	3R	6.56	7.13	0.57	-	-	-	-
		3N	6.59	6.91	0.32	-	-	-	-
4	upstream	4R	6.33	6.58	0.25	-	-	-	-
		4N	6.53	6.84	0.31	-	-	-	-
5	downstream	5R	6.60	7.52	0.92	-	-	-	-
		5N	6.59	7.02	0.43	-	-	-	-
6	upstream	6R	6.62	7.03	0.41	-	-	-	-
		6N	6.62	7.24	0.62	-	-	-	-
7	downstream	7R	6.57	7.43	0.86	-	-	-	-
		7N	6.86	7.45	0.59	-	-	-	-
8	upstream	8R	6.60	7.01	0.41	-	-	-	-
		8N	6.59	7.62	1.03	-	-	-	-
9	downstream	9R	6.59	7.29	0.70	-	-	-	-
		9N	6.56	7.34	0.78	-	-	-	-
10	upstream	10R	6.63	7.73	1.10	-	-	-	-
		10N	6.58	7.78	1.20	-	-	-	-

Note: Gerbils were placed on false bottom at 9:00 (no time recorded on lab book) am.

Table 7. 19: Trophozoite detection by samples from KL on Month: 08 Day: 06/07Year: 2003
Filtration Date: July 21,22/2003 Infection Date: July 25/2003

Cage#	Sample Source	Tube#	Tube# Continue					
			A		B		C	D
			Count1	Count2	Count1	Count2		
1	downstream	1R	-	-	-	-	-	-
		1N	-	-	+	-	-	+
2	upstream	2R	++	++	-	-	++	-
		2N	++	++	++	++	-	-
3	downstream	3R	-	-	-	-	-	-
		3N	-	-	-	-	-	+
4	upstream	4R	++	++	-	-	-	-
		4N	++	++	-	-	-	-
5	downstream	5R	-	-	-	-	-	-
		5N	-	-	-	-	-	-
6	upstream	6R	-	-	-	-	-	++
		6N	++	++	-	-	-	-
7	downstream	7R	-	-	-	-	-	-
		7N	-	-	-	-	-	-
8	upstream	8R	+++	+++	+++	+++	-	-
		8N	++	++	-	-	-	-
9	downstream	9R	-	-	-	-	-	-
		9N	-	-	-	-	-	-
10	upstream	10R	-	-	-	-	-	-
		10N	+	+	++	++	-	-

Note: Downstream was checked on Aug. 06/2003, upstream was checked on Aug.07/2003.

+: Trophozoites observed were dead, no flagella movement.
++: Positive, Flagella moving,
+++:K8R-A very positive sample, trophozoites extremely mobile(appear translucent) heavily infected gerbil, same as K8R-B

Table 7. 20: *Giardia lamblia* cysts detection by samples from KL on Month: 07 Day: 07Year: 2003
Filtration Date: June 21,22/2003 Infection Date: June 24/2003

Cage #	Sample source	Tube #	Weight 1	Weight 2	∇ Weight	Count 1	Count 2	Count 3	Count 4
1	downstream	1R	6.47	7.27	0.80	-	-	-	-
		1N	6.47	7.44	0.97	-	-	-	-
2	upstream	2R	6.51	7.49	0.98	-	-	-	-
		2N	6.54	7.37	0.83	-	-	-	-
3	downstream	3R	6.43	7.06	0.63	-	-	-	-
		3N	6.37	7.18	0.81	-	-	-	-
4	upstream	4R	6.47	7.29	0.82	-	-	-	-
		4N	6.44	7.41	0.97	-	-	-	-
5	downstream	5R	6.70	7.73	1.03	-	-	-	-
		5N	6.61	7.62	1.01	-	-	-	-
6	upstream	6R	6.65	7.17	0.52	-	-	-	-
		6N	6.48	7.38	0.90	-	-	-	-
7	downstream	7R	6.55	7.42	0.87	-	-	-	-
		7N	6.74	7.61	0.87	-	-	-	-
8	upstream	8R	6.44	7.21	0.77	-	-	-	-
		8N	6.43	7.18	0.75	-	-	-	-
9	downstream	9R	6.49	7.32	0.83	-	-	-	-
		9N	6.52	7.49	0.97	-	-	-	-
10	upstream	10R	6.47	7.31	0.84	-	-	-	-
		10N	6.61	7.79	1.18	-	-	-	-

Note: Gerbils were placed on false bottom at 8:30am and feces were collected in 1 hour (6:00am) (The original feces collected time is 9:30am was changed by the technician).

Table 7. 21: *Giardia lamblia* cysts detection by samples from KL on Month: 07 Day: 08Year: 2003
Filtration Date: June 21,22/2003 Infection Date: June 24/2003

Cage #	Sample source	Tube #	Weight 1	Weight 2	∇ Weight	Count 1	Count 2	Count 3	Count 4
1	downstream	1R	6.79	7.21	0.42	-	-	-	-
		1N	7.03	7.83	0.80	-	-	-	-
2	upstream	2R	6.58	7.23	0.65	+*	-	-	-
		2N	6.78	7.11	0.33	-	-	-	-
3	downstream	3R	7.11	7.94	0.83	-	-	-	-
		3N	7.01	7.61	0.60	-	-	-	-
4	upstream	4R	6.80	7.44	0.64	-	-	-	-
		4N	6.51	7.51	1.00	-	-	-	-
5	downstream	5R	6.92	7.12	0.20	-	-	-	-
		5N	7.02	7.76	0.74	-	-	-	-
6	upstream	6R	7.02	7.81	0.79	-	-	-	-
		6N	6.84	7.24	0.40	-	-	-	-
7	downstream	7R	6.56	7.14	0.58	-	-	-	-
		7N	6.49	6.99	0.50	-	-	-	-
8	upstream	8R	6.78	7.15	0.37	-	-	-	-
		8N	7.08	7.71	0.63	-	-	-	-
9	downstream	9R	7.00	7.51	0.51	-	-	-	-
		9N	6.80	7.09	0.29	-	-	-	-
10	upstream	10R	6.79	7.09	0.30	-	-	-	-
		10N	6.82	7.15	0.33	-	-	-	-

Note: Gerbils were placed on false bottom at 7:20am and this time was changed from 8:15 from technician's lab book. There is no feces collected time available.

+*: Similar shape as *Giardia Lamblia* cyst, but has glossy outer surface. Most likely glove contamination (no organelles)

Table 7. 22: Trophozoite detection by samples from KL on Month: 07 Day: 09/10Year: 2003
Filtration Date: June 21,22/2003 Infection Date: June 24/2003

Cage#	Sample Source	Tube#	Tube# Continue					
			A		B		C	D
			Count1	Count2	Count1	Count2		
1	downstream	1R	-	-	-	-	-	-
		1N	-	+	-	-	-	-
2	upstream	2R	-	-	-	-	-	-
		2N	-	-	-	-	-	-
3	downstream	3R	-	-	-	-	-	-
		3N	-	-	-	-	-	-
4	upstream	4R	-	-	++	-	-	-
		4N	++	-	-	++	-	-
5	downstream	5R	+	-	-	-	-	-
		5N	-	-	+	-	-	-
6	upstream	6R	-	-	-	-	-	-
		6N	-	-	-	-	-	-
7	downstream	7R	-	-	-	-	-	-
		7N	-	-	-	-	-	-
8	upstream	8R	++	++	++	++	-	-
		8N	-	-	++	++	-	-
9	downstream	9R	-	-	-	-	-	-
		9N	-	-	-	-	-	-
10	upstream	10R	++	++	++	++	-	-
		10N	++	++	++	++	-	-

Note: Downstream was checked on July 09/2003, upstream was checked on July10/2003.

+: Parasites observed were dead, no movement of flagella, observed by Dr. Belosevic (downstream only).
++: Parasites observed with flagella movement.

7.1.3 Information required for Filtration

Table 7. 23: Information required for filtration set #1 at City Edmonton:

Filtration order	Sample location	Meter reading		Total V.(L)	Time consume		Pressure Meter(psi)		Flow rate L/min	
		Start	End		Start at	End at	Start at	End at	Start at	End at
1	Downstream	7460	7610	150	9:40	10:20	30	38.5	3.4	3
2	Downstream	7610	7760	150	10:25	11:15	38.5	42.5	3.6	2.6
3	Upstream	7760	7910	150	11:25	12:15	38.5	52	3.6	2
4	Upstream	7910	8060	150	12:25	13:30	31	47.5	3.4	1.7

Filtration was processed on June 10th 2003

Table 7. 24: Information required for UV Reactor at City Edmonton for filtration set #1:

UV Reactor Model	UV 4000 series (1998)	
UV lamps Manufacturer or Supplier	Trojan Industries	
Number of Lamps Per Channel	In total	180 lamps
	In operation	180 lamps
Number of Channels	In total	4
	In operation	2
Physical dimension for reactor	Length	2.0 m
	Width	1.05 m
	Height	1.15 m
Lamps power		
Lamps age	T.B.D.	
Sensor reading		
Calculated dosage	Est.30 mJ/cm^2	

Table 7. 25: Information required for wastewater at City Edmonton for filtration set #1:

Flow rate	Upstream UV	
	Downstream UV	
Total suspended solid Mg/l(TSS)	(unofficial) 5 mg/L by GoldBar Laboratory	
Total coliform CFU/100ml	Upstream UV	
	Downstream UV	(unofficial) 430 (fecal coliform 8)
Transmittance %-10mm cell, 254nm	65	
Brief description of treatment process		

Table 7. 26: Information required for filtration set #2 at City Edmonton:

Filtration order	Sample location	Meter reading		Total V.(L)	Time consume		Pressure Meter(psi)		Flow rate L/min	
		Start	End		Start at	End at	Start at	End at	Start at	End at
1	Downstream	8590	8740	150	9:10	9:55	30	40	4	3
2	Downstream	1292	1442	150	9:15	9:58	30	40	4	3
3	Upstream	8740	8890	150	10:05	10:55	30	40	4	3
4	Upstream	1442	1592	150	10:05	10:55	30	40	4	3

Filtration was processed on August 12th 2003.

Table 7. 27: Information required for UV Reactor at City Edmonton for filtration set #2:

UV Reactor Model	UV 4000 series (1998)	
UV lamps Manufacturer or Supplier	Trojan Industries	
Number of Lamps Per Channel	In total	180 lamps
	In operation	180 lamps
Number of Channels	In total	4
	In operation	2
Physical dimension for reactor	Length	2.0 m
	Width	1.05 m
	Height	1.15 m
Lamps power		
Lamps age	T.B.D.	
Sensor reading		
Calculated dosage	Est.30 mJ/cm^2	

Table 7. 28: Information required for wastewater at City Edmonton for filtration set #2:

Flow rate	Upstream UV	
	Downstream UV	
Total suspended solid Mg/l(TSS)	(unofficial) 5 mg/L by GoldBar Laboratory	
Total coliform CFU/100ml	Upstream UV	
	Downstream UV	(unofficial) 430 (fecal coliform 8)
Transmittance %-10mm cell, 254nm	61	
Brief description of treatment process		

Table 7. 29: Information required for filtration set #3 at City Edmonton:

Filtration order	Sample location	Meter reading		Total V.(L)	Time consume		Pressure Meter(psi)		Flow rate L/min	
		Start	End		Start at	End at	Start at	End at	Start at	End at
1	Downstream	9080	9230	150	9:05	10:15	35	50	4	1
2	Downstream	1790	1940	150	9:10	10:20	35	50	4	1
3	Upstream	9230	9380	150	10:20	11:25	25	50	3	1.5
4	Upstream	1950	2090	140	10:25	11:40	25	50	3	0.5
5	Spike	9380	9530	150	11:30	12:45	25	55	3.4	0.5
6	Spike	2090	2240	150	11:45	13:20	25	55	4	0.5

Filtration was processed on August 26th 2003.

Table 7. 30: Information required for UV Reactor at City Edmonton for filtration set #3:

UV Reactor Model	UV 4000 series (1998)	
UV lamps Manufacturer or Supplier	Trojan Industries	
Number of Lamps Per Channel	In total	180 lamps
	In operation	180 lamps
Number of Channels	In total	4
	In operation	2
Physical dimension for reactor	Length	2.0 m
	Width	1.05 m
	Height	1.15 m
Lamps power		
Lamps age	T.B.D.	
Sensor reading		
Calculated dosage	Est.30 mJ/cm^2	

Table 7. 31: Information required for wastewater at City Edmonton for filtration set #3:

Flow rate	Upstream UV	
	Downstream UV	
Total suspended solid Mg/l(TSS)	(unofficial) 5 mg/L by GoldBar Laboratory	
Total coliform CFU/100ml	Upstream UV	
	Downstream UV	(unofficial) 430 (fecal coliform 8)
Transmittance %-10mm cell, 254nm	65	
Brief description of treatment process		

Table 7. 32: Information required for filtration set #1 at City Kelowna:

Filtration order	Sample location	Meter reading		Total V.(L)	Time consume		Pressure Meter(psi)		Flow rate L/min	
		Start	End		Start at	End at	Start at	End at	Start at	End at
1	Upstream	7189	7850	661	10:00	12:15	42	48	7	3
2	Downstream	7850	8450	600	12:35	14:55	46	51	7	3
3	Downstream	8450	9070	620	9:35	11:35	41	46	6	3
4	Upstream	9070	9660	590	12:05	14:40	39	45	6	3

Note: 1. Sample 1 and 2 were filtered on June 21/03, sample 3,4 were filtered on June 22/03

2. There was a 15 min sample water rinse between each sample.

Table 7. 33: Information required for UV Reactor at City Kelowna for filtration set #1:

UV Reactor Model	Trojan UV 3000 series	
UV lamps Manufacturer or Supplier	LSI Trojan Industries	
Number of Lamps Per Channel	In total	576 lamps
	In operation	576 lamps (Max) 192lamps (Min)
Number of Channels	In total	2
	In operation	2
Physical dimension for reactor	Length	40feet
	Width	5feet
	Height	4feet
Lamps power	100 watts /lamp	
Lamps age	T.B.D. Varies from 300-15,000	
Sensor reading	Avg. 3 milli watts/cm^2	
Calculated dosage	50,000 microwatt seconds/cm^2	

Table 7. 34: Information required for wastewater at City Kelowna for filtration set #1:

Flow rate	Upstream UV	200-700 L/S
	Downstream UV	200-700 L/S
Total suspended solid Mg/l(TSS)	0.9 mg/L (Avg. 2002)	
Total coliform CFU/100ml	Upstream UV	234,152 2002 avg. (idexx in house)
	Downstream UV	2.9 2002 avg. (membrane filtration)
Transmittance %-10mm cell, 254nm	60.3%T(based on a 0.220/cm UV absorbance @ 254nm)	
Brief description of treatment process	Primary Clarification followed by a Barden pho nutrient removal bioreactor dual media gravity filtration and UV disinfection	

Table 7. 35: Information required for filtration set #2 at City Kelowna:

order	Sample location	Meter reading		Total V.(L)	Time consume		Pressure Meter(psi)		Flow rate L/min	
		Start	End		Start at	End at	Start at	End at	Start at	End at
1	Upstream	80004	80538	534	9:20	12:20	50	62	6	2
2	Downstream	80538	81138	600	12:55	14:55	54	64	6	3
3	Downstream	81146	81712	566	8:30	11:02	49	58	6	3
4	Upstream	81714	82382	668	11:20	14:40	46	58	6	2

Note: 1. Sample 1 and 2 were filtered on July 21/03, sample 3,4 were filtered on July 22/03

2. There was a 15 min sample water rinse between each sample.

Table 7. 36: Information required for UV Reactor at City Kelowna for filtration set #2:

UV Reactor Model	Trojan UV 3000 series	
UV lamps Manufacturer or Supplier	LSI Trojan Industries	
Number of Lamps Per Channel	In total	576 lamps
	In operation	576 lamps (Max) 192lamps (Min)
Number of Channels	In total	2
	In operation	2
Physical dimension for reactor	Length	40feet
	Width	5feet
	Height	4feet
Lamps power	100 watts /lamp	
Lamps age	T.B.D. Varies from 300-15,000	
Sensor reading	Avg. 3 milli watts/cm^2	
Calculated dosage	50,000 microwatt seconds/cm^2	

Table 7. 37: Information required for wastewater at City Kelowna for filtration set #2:

Flow rate	Upstream UV	200-700 L/S
	Downstream UV	200-700 L/S
Total suspended solid Mg/l(TSS)	0.9 mg/L (Avg. 2002)	
Total coliform CFU/100ml	Upstream UV	234,152 2002 avg. (idexx in house)
	Downstream UV	2.9 2002 avg. (membrane filtration)
Transmittance %-10mm cell, 254nm	N/A	
Brief description of treatment process	Primary Clarification followed by a Barden pho nutrient removal bioreactor dual media gravity filtration and UV disinfection	

Table 7. 38: Information required for filtration set #3 at City Kelowna:

order	Sample location	Meter reading		Total V.(L)	Time consume		Pressure Meter(psi)		Flow rate L/min	
		Start	End		Start at	End at	Start at	End at	Start at	End at
1	Downstream	82482	83135	653	8:20	11:20	32	36	6	3
2	Upstream	83135	83740	605	11:35	14:40	32	38	6	2.5
3	Downstream	83740	84480	740	8:40	11:32	48	56	6	3
4	Upstream	84480	85120	640	11:50	14:40	54	62	6	3

Note: 1. Sample 1 and 2 were filtered on Aug. 18/03, sample 3,4 were filtered on Aug. 19/03

3. There was a 15 min sample water rinse between each sample.

Table 7. 39: Information required for UV Reactor at City Kelowna for filtration set #3:

UV Reactor Model	Trojan UV 3000 series	
UV lamps Manufacturer or Supplier	LSI Trojan Industries	
Number of Lamps Per Channel	In total	576 lamps
	In operation	576 lamps (Max) 192lamps (Min)
Number of Channels	In total	2
	In operation	2
Physical dimension for reactor	Length	40feet
	Width	5feet
	Height	4feet
Lamps power	100 watts /lamp	
Lamps age	T.B.D. Varies from 300-15,000	
Sensor reading	Avg. 3 milli watts/cm^2	
Calculated dosage	50,000 microwatt seconds/cm^2	

Table 7. 40: Information required for wastewater at City Kelowna for filtration set #3:

Flow rate	Upstream UV	200-700 L/S
	Downstream UV	200-700 L/S
Total suspended solid Mg/l(TSS)	0.9 mg/L (Avg. 2002)	
Total coliform CFU/100ml	Upstream UV	234,152 2002 avg. (idexx in house)
	Downstream UV	2.9 2002 avg. (membrane filtration)
Transmittance %-10mm cell, 254nm	N/A	
Brief description of treatment process	Primary Clarification followed by a Barden pho nutrient removal bioreactor dual media gravity filtration and UV disinfection	

7.2 Reagents Preparation

7.2.1 IMS reagents supply

Table 7. 41: IMS reagents supply

Name of Product	Cat. #	Quality and Quantity	Supplier	Price
Pre-separation Column	130-041-407	50/per package	Miltentiliotec 12740 Earhart Avenue Auburn, CA 95602 USA Ph: 530-888-8871 (800)FORMACS FAX: 530-888-8925	
Separation Column	130-042-201	25/per pachage	The same as above	
Anti_cryptos-poridium antibody	LR50	1ml/per vial	Immucell Corporation 56 Evergreen Drive Portland, ME 04103 Ph:1-800-466-8235 www.immucell.com	200./ea
FITC labeled goat anti-mouse	12064D	0.5 mg/ml	Farmingen A Becton Dickinson Co. Ph: 1-888-259-0187 www.bdbiosciences.com	231./ per vial can get 5% discount
Microbeads	130-048-701		Miltentiliotec 12740 Earhart Avenue Auburn, CA 95602 USA Ph: 530-888-8871 (800)FORMACS FAX: 530-888-8925	
New Calf Serum	511250	500ml	Atlanta Biologicals 1425 OAIC Brook Drive Suite 400 Norcross GA 30093 Ph: 1-800-780-7788	34/ea

7.3 Cultivation of Giardia lamblia

7.3.1 Materials

Giardia lamblia (W.B. Strain) trophozoits from –80°C freezer

Disposable polystyrene flasks: 250 cm^2 and 75 cm^2 canted neck, tissue culture flasks with plug seal cap

Diamond's TYI-S-33 medium:	**500 ml**	**1L**
Trypticase-peptone	10 g	20 g
Yeast extract	5.0 g	10 g
Dextrose	5.0 g	10 g
Sodium chloride	1.0 g	2 g
L-cysteine hydrochoric acid	1.0 g	2 g
Ascorbic acid	0.1 g	0.2 g
K2HPO4	0.5 g	1 g
KH2PO4	0.3 g	0.6 g
Ferric ammonium citrate	0.01 g	0.02 g
Calf serum (3 months old-Hyclone lab)	50 ml	100 ml
Vitamin additive (NCTC-13 from Gibco-BRL)	15 ml	30 ml
Gentamycin (50 mg/L from Gibco-BRL)	1 ml	2 ml
MilliQ water up to:	500 ml	1 L

Adjust pH to 6.8 with sodium hydroxide.

Filter medium through 0.8 (Milli-Fil- P.F. Millipore Corp.) and 0.22 um filters (Sterivex-GS filters with filling bell, Millipore Corp.) at a peristaltic pump speed of 1.9.

7.3.2 Encystation medium

Diamond's TYI-S-33 medium

with 10 mg/ml bovine bile (4 g/ 400 ml) and 0.5 mM lactic acid (0.022 g/ 400 ml)

The procedure only requires 250 ml but so much is lost in filtration so start with 400 ml.

Adjust the pH 7.8

Filter through Membrane filter and 0.8 and 0.22 um filters.

7.3.3 Methods

Use aseptic techniques and work in a fume hood for all methodology.

Day 1

Obtain the *G. lamblia*, W.B. strain from the –80°C freezer and thaw at 37°C right before transferring it into a 75 cm^2 canted neck tissue culture flask filled with Diamond's TYI-S-33 medium.

Close the flask with a plug and seal with parafilm wax. Place in a 37°C incubator.

Day 2

After the trophozoits have grown to confluence (when they cover the bottom of the flask – this may take a day or two) invert the flask several times and empty the spent growth medium with the unattached trophozoits. Then fill the flask with encystation medium.

Incubate the culture at 37°C for 24 hours.

Day 3

Place the culture on ice for 10 minutes. Slap the sides to dislocate the cysts. Transfer 500 ml into 5 or 6, 50 ml centrifuge tubes. Centrifuge at 400 g (1450 rpm on Jouan) for 10 minutes. Pour off encystation media and replace it with 5 ml freshly prepared Diamond's TYI-S-33 medium. Agitate tubes and pour into a large flask. Top it off with growth media

7.4 Protocol for Giardia and Crypotospridium Sampling at WWTPs

7.4.1 Purpose of sampling

The wastewater sampled both at upstream and downstream of UV reactors at wastewater treatment plants by Filta-Max™ filters will be used on the research regards about the ability of infectivity of *Giardia Lamblia* and *Crypotospridium parvum* by animal model.

Concentrated wastewater sample will be diluted and purified at lab. Final sample which contain sufficient number of *Giardia Lamblia* and *Crypotospridium parvum* will be used to infect Mongolian Gerbils to test the ability of infectivity of *Giardia Lamblia* and *Crypotospridium parvum.*

7.4.2 Component of sampling apparatus

The sampling apparatus consists of: tube, pump, Pressure g auge, Filta-max™ housing and filter, a short piece of Tygon tubing connected to an ABB water metre, and a

garden hose to drain the system. Plugged Filter housings will be placed in Zip Loc plastic bags and transported in coolers. Filters will be stored at 4°C until elution processing.

7.4.3 Procedure of Sampling

The recommended operation pressure of the filter housing is 5 bar (75psi). Do not exceed the maximum operation pressure of 8 bar (120psi). The operation flow rate should be controlled between 1-4L/min. Sampling stop at the filters clogged or the flow rate changes apparently.

Sample should be taken at the middle height and middle width of inlet channel and outlet channel.

Four filters will be sent to sampling site which two for UV upstream and two for UV downstream. The order for sampling should be taken as one at down stream then one at upstream respectively.

The whole sample system should be rinsed thoroughly when change sample site from upstream to downstream before install Filta-Max™ filters to avoid the cross contamination.

Two liters water bottles in which will be filled by upstream water will be provided too. Plugged filter housings will be placed in Zip loc plastic bags and transported along with water bottles in coolers by courier.

In order to infect animal on Friday, filtration should start on Monday and send sample out by courier on Tuesday.

Filters, filter housings, coolers and package materials will be provided by University of Alberta.